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DOCTOR OF PHILOSOPHY

The investigation of pyrolysis products of South African street drug, nyaope

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**The investigation of pyrolysis products of South African  
street drug nyaope**

**By**

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**Supervisor: Professor Niamh Nic Daéid**

***A thesis submitted to the University of Dundee towards the  
fulfilment of the requirements for the degree of Doctor of  
Philosophy***

**March 2018**

#### Statement of declaration

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I hereby state that I am the author of this thesis and that, unless otherwise stated, all references cited have been consulted by me; that the work of which the thesis is a record has been done by me, and that it has not been previously accepted for a higher degree.

Signed: \_\_\_\_\_

 *Prof. N. N. D. Arora*

## Dedications

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*To my son Khotso Dennis Phokedi with all my love*

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## Abstract

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This research investigates a new South African street drug called nyaope. It is not exactly known what the ingredients of this drug are, but some reports say it is a cocktail of many illegal substances ranging from heroin, cannabis, methamphetamine, cocaine to pain killers, where in each case, the material also includes anti-retroviral drugs. The main ingredient is believed to be the anti-retroviral drug, efavirenz (EFV), thought to prolong the effects of other narcotic drugs in the nyaope mixture. Following reports of an overwhelming increase of its abuse, nyaope was eventually brought under legal control in 2014. Originally nyaope was mixed with cannabis for smoking but more recently people also choose to chew, snort, inject or heat the mixtures over aluminium foil and inhale the fumes.

The work presented in this thesis involves the first development and validation of a methodology for the routine isolation of pyrolysis products of efavirenz in mixtures with other alleged components of the nyaope street drug. The products (generated during pyrolysis) can be used to study the abuse potential of efavirenz in terms of a verification of its presence or otherwise in nyaope residues seized by law enforcement. Pyrolysis products were analysed using Gas Chromatography Mass Spectrometry (GCMS) and High Performance Liquid chromatography (HPLC).

Pyrolysis products of EFV on its own and then in mixtures with other suggested components of the nyaope street drug (methamphetamine, amphetamine, heroin, cannabis and opium) were sequentially investigated. The successful use of activated carbon strips in trapping pyrolysis products of the various illicit drugs and drug mixtures during this research was demonstrated and a novel robust analytical methodology developed.

Each drug was first heated separately and analysed to generate their background pyrolysis product signature. This was followed by mixing each drug with efavirenz and heating the mixture. The results revealed that the presence of efavirenz in mixtures of Amphetamine type stimulant drugs and heroin did not disrupt the production of the expected pyrolysis products of these drugs and vice versa.

However, heating a mixture of opium and EFV revealed only the presence of EFV and its pyrolysis product suggesting that the presence of EFV prevented the pyrolysis of the cannabis and opium.

The pyrolysis of methamphetamine and amphetamine interestingly, also revealed the presence of synthetic reaction impurities from starting materials and intermediate products of chemical reactions applied during the various synthesis processes. These were further examined and were successfully used to determine the potential synthetic routes used during the manufacture of the methamphetamine samples used for this research. This provides new opportunities to generate intelligence information regarding the manufacturing process of these materials from physical evidence such as residues of smoked or heated materials.

## Thesis Overview

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Chapter 1 discusses the global challenges regarding illegal substances as well as information (in figures) supplied by the United Nations (UN) on numbers of people estimated as being affected by the illicit drug trade over the years. Also presented is the summary of progress made by the UN, throughout the years, in the fight against drugs. World drug laws including measures put in place by different countries towards the curbing of drugs are also discussed. The worsening drug situation in South Africa including challenges caused by the new South African street drug, nyaope, is discussed in detail. Also included in this chapter are the objectives of this research.

Chapter 2 discusses the principles and applications of the analytical techniques used during this research. Two chromatographic techniques; Gas Chromatography Mass Spectroscopy (GCMS) and High Performance Liquid Chromatography (HPLC), were used during this research. The discussion also includes individual components of each instrument and their role in achieving the desired results.

In chapter 3, the development and validation of the analysis method used for this research is discussed in detail. This includes the procedure for testing column performance to ensure repeatability of results, instrument calibration and other work necessary for proper validation. Also covered is the optimisation of the Activated Carbon Strip (ACS) method for the extraction of the pyrolysis products of EFV and other drugs from a drug mixture sample.

Chapter 4 discusses two illegal synthetic drugs (amphetamine and methamphetamine), alleged to be part of the ingredients used in the nyaope mixture. We also investigate how the presence of the target analyte, efavirenz (EFV), (alleged to be main ingredient of the drug mixture) influences the pyrolysis of each illicit drug and vice versa. This is achieved through the comparison of analytical results obtained when EFV was first heated on its own and when it was heated in mixtures with other alleged components of the nyaope.



Chapter 5 discusses three illegal drugs which are derived from plants (cannabis, opium and heroin) and alleged to be part of the ingredients used in the nyaope mixture. The influence of the EFV on the pyrolysis of these three drugs is investigated and discussed.

Overall conclusions of this research are discussed in Chapter 6 including suggestions for future work regarding the analysis of pyrolysis products of the nyaope street drug mixture.

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## List of abbreviations

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°C	degrees Celsius
µg	micrograms
µg/mL	micrograms per millilitre
µL	microlitre
ACN	acetonitrile
ACS	activated carbon strip
AIDS	acquired immune deficiency syndrome
ART	anti-retroviral therapy
ARV	anti-retro viral
ASTM	American Society for Testing and Materials
ATS	amphetamine type stimulant
CBC	cannabichromene
CBD	cannabidiol
CBG	cannabigerol
CBN	cannabinol
CI	chemical ionisation
CONC	concentration
CuSO <sub>4</sub>	copper sulphate
DAD	diode array detector
DALYs	disability adjusted life years
DC	direct current
DCM	dichloromethane
DNA	deoxyribonucleic acid
EFV	efavirenz
EFV 1	efavirenz pyrolysis product 1

EI	electron ionisation
GC	gas chromatography
GCMS	gas chromatography mass spectrometry
g	grams
g/mol	grams per mole
H	hydrogen
HCl	hydrogen chloride
HCOOH	formic acid
HI	hydrogen iodide
HIV	human immune deficiency virus
HIV-1RT	human immunodeficiency virus type 1
HPLC	high performance liquid chromatography
ICH	international conference on harmonisation
i.d	internal diameter
INCB	international narcotics control board
ISO/IEC	International Organization for Standardization and the International Electrotechnical Commission
KOH	potassium hydroxide
KH <sub>2</sub> PO <sub>4</sub>	potassium dihydrogen phosphate
Li	lithium
LOD	limit of detection
LOQ	limit of quantification
LSD	lysergic acid diethylamide
m	meter
mg	milligram
min	minute
mL	millilitre

mm	millimetre
mg/mL	milligrams per millilitre
mL/mn-1	millilitres per minute
MeOH	methanol
m/z	mass to charge ion
Na	sodium
Na <sub>2</sub> HPO.12H <sub>2</sub> O	Disodium hydrogen phosphate dodecahydrate
NGO	non-governmental organisation
NH <sub>3</sub>	ammonia
NIST	national institute of standards and technology
nm	nanometres
Pd/C	palladium on carbon
PFTE	Polytetrafluoroethylene
P2P	1-phenyl-2-propanone
Puff	the act of drawing smoke out of a cigarette, inhaling then exhaling it
PMCT	prevention of mother to child transmission
PWID	people who inject drugs
R <sup>2</sup>	coefficient of determination in regression
RSD	relative standard deviation
RT	retention time
SANBS	South African national blood services
SCOT	support coated open tubular
SD	standard deviation
SIM	selective ion monitoring
Std	standard
Standard amount	amount of reference material used

TDM	therapeutic drug monitoring
TIC	total ion monitoring
THC	tetrahydrocannabinol
$\Delta^9$ THC	delta-9-tetrahydrocannabinol
UN	United Nations
UNAIDS	United Nations programme on HIV/AIDS
UNODC	United Nations office on drugs and crimes
UNCITNAPS	United Nations conventions on illicit trafficking of narcotics and psychotropic substances
UV	ultra violet
UV-VIS	ultra violet-visible
v/v	volume by volume
WCOT	wall coated open tubular
WDR	world drug report
WHO	world health organisation

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## **CHAPTER 1: INTRODUCTION**

### **1.1 World drug situation and war on drugs**

According to the 2017 World Drug Report (WDR), an estimated quarter of a billion or (5%) of the global adult population used controlled drugs at least once in the year 2016. The report further states that about 29.5 million (0.6%) of that number suffer from drug use disorders [1]. The magnitude of the harm caused by drug use is underlined by the estimated 28 million years of 'healthy' life (disability –adjusted life years (DALYs)) lost worldwide in 2015 as a result of premature death and disability caused by drug use. Amongst those, 17 million are wholly attributed to drug use disorders across all drug types. DALYs attributable to morbidity and mortality due to drug use have been observed to have increased in the past decade [1].

Different countries and different regions of the world face different illicit drug challenges; in general drug sales, possessions and use are regarded as illegal by most countries [2-5]. Substance abuse and deterioration of personal health cannot be separated and both have become a common topic addressed by both the United Nations (UN) and the World Health Organisation (WHO). Psycho-active substances cause significant health and social problems for the people who use them, and also for others in their community [6-8]. Policies which influence the levels and patterns of substance use and related harm can significantly reduce public health issues caused by substance abuse [8]. The WHO seeks to promote the rehabilitation and treatment of the affected individuals by challenging countries to generate interventions at health care system levels that can work towards the restoration of health in affected individuals. The organisation insists that it has strategies to help reduce the burden of psychoactive substance use and is committed to assisting countries with the development, organisation, monitoring and evaluation of treatment and other services [7, 8].

The UN promotes the policy of bringing the illegal drugs market under control through the punishment of drug traffickers and provision of treatment and rehabilitation to those affected. As a result, member states are challenged to adopt

the developed policies. The approach has since been adopted and adapted by most European countries where both legislation and resources for rehabilitation and education are in place [6-8].

Law enforcement in most countries is focused towards the punishment of drug dealers and traffickers rather than making use by an end user a criminal offence. This approach however does not seem to bring the situation under control because the number of drug users continue to grow [1]. Many countries have spent, and continue to spend, funds to put resources in place towards public education against drug use, rehabilitation of those affected as well as on their medical care [4].

Scientists are mandated to continue their support for the war against drugs through research; to come up with strategies for the detection and identification of emerging drugs.

## **1.2 History of the United Nations 'on war' against drugs**

The UN suggests the use of proper legislation as one of the many solutions towards counteracting the spread of illicit drugs, and has been involved with drafting and the adoption of international treaties for the control of drugs since the early 1900s.

The International Opium Conference held in Shanghai in 1909 is seen as the first international effort to control illicit drugs [9]. The forum had initially met to exchange data and information on the illegal sale, consumption and production of opium in Asia, but the topic was later extended to include other countries as well. As this was a commission, participants could not make binding international agreements but were able to recommend actions necessary to prevent the trafficking of opium and its abuse. Resolutions passed urged national governments to make the necessary legislation to curb opium smoking in their countries, initiate the regulation of opium for non-medical purposes, ban opium exportation to countries that prohibited its importation and control the manufacture and distribution of opium derivatives [9].

The commission was the first effective step taken by the international community to combat drug abuse. It served as a catalyst for countries to pass domestic legislation addressing drug problems within their borders. Most importantly, the commission united countries in an international cooperative effort to address the problem of the opium trade. The work of the commission led to the convening of the Hague Opium Conferences (1912-1914) and to the adoption of the 1912 International Opium Convention, sometimes called the Hague Opium Convention, and succeeding treaties that effectively restricted opium production and trade to legitimate purposes [10].

Resolutions from the Shanghai conference were used as the basis for the first international drug convention, The International Opium Convention of The Hague signed in 1912 and entered into effect in 1915 [10]. The convention banned the production and shipment of opium and its derivatives except those used for medicinal purposes. The Peace Treaty of Versailles signed on the 28<sup>th</sup> June, 1919 (Paris) supported the convention by including a clause that required all its signatories to adhere to the provisions of the International Opium Convention of The Hague [10].

As of 1920, issues regarding international drug control became part of the tasks assumed by the League of Nations, a predecessor of the United Nations. Under the auspices of the League, three main conventions were developed (1925 Convention, 1931 Convention and 1936 Convention). The three conventions provided the groundwork for the practical operations of an international drug control system which resulted in considerable progress being made towards the reduction of licit trade in narcotic drugs.

The League of Nations, established after the World War I, took over as the custodian of the Opium convention. This convention was later upgraded and signed in 1925, and over the following years the signing of conventions for limiting the manufacturing and distribution of narcotics only to medical and scientific purposes, the adoption of legal documents to allow certain drugs to become international

offences were achieved [10]. Also accomplished were the transfer of The League of Nations to the United Nations (1945) and the assignment of the Commission of Narcotic Drugs [11] as the central policy making body of the United Nations drug related matters in 1946. Many substances were later placed under international control by the Synthetic Narcotics Protocol (1948) [12]. The opium protocol (1953) also ensured that opium production and trade were also only limited to scientific and medicinal needs [12].

In 1961, the Single Convention on Narcotic Drugs was adopted as the first international treaty used to control the manufacture and trafficking of narcotic drugs [13]. The convention consolidated and broadened previous treaties to include cannabis and also ensured that drugs with similar effects to those specified in the convention were listed as controlled. It empowered the Commission on Narcotic Drugs and the World Health Organisation to add, remove, and transfer drugs among the treaty's four schedules of controlled substances. It also ensured the establishment of the International Narcotics Control Board (INCB). The board was mandated with the administration of issues relating to drug control and production, International trade, and dispensation [13]. This treaty was supplemented by both the Convention on Psychotropic Substances 1971, which ensured the control of LSDs, ecstasy, and other psychotropic pharmaceuticals and the United Nations Convention against Illicit Trafficking in Narcotic Drugs and Psychotropic Substances (UNCITNAPS), which strengthens provisions against money laundering and other drug related offences [14].

During the 1960s drug use increased greatly around the world, especially in Western nations. Inspired by psychedelic advocates like Aldous and Timothy Leary, millions of people experimented with powerful hallucinogens and many drugs of all kinds became more freely accessible [14]. The Single Convention of 1961 failed to ban many newly discovered drugs because its scope was only limited to drugs with similar effects such as cannabis, coca and opium. The Convention on Psychotropic Substances 1971 was therefore introduced, ensuring the control of psycho active drugs such as amphetamines, barbiturates and psychedelics [14].

In 1988 United Nations Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances was introduced to include the newly discovered drug compounds. This is one of the three major drug control treaties currently in force. It provides additional legal for enforcing the 1961 Single Convention on Narcotic Drugs and the 1971 Convention on Psychotropic Drugs [14].

In 1997, the United Nations Office on Drugs and Crime (UNODC) was also established and made responsible for monitoring of drugs situation in each country and to work with national authorities at ensuring compliance with the Single Convention.

All these efforts by the United Nations to fight illicit drugs have since become a foundation through which countries develop and adopt strategies to address the challenge of illegal drugs. The scope of control of drugs has broadened over the years; from opium to cocaine to cannabis to psychotropic substances, including the regulation of production of trade of medical drugs; the international cooperation to fight complex problems associated with illicit drugs is now a reality. The legal framework for the whole international drug control system is now based on these three international conventions (1961, 1971 and 1988).

### **1.3 Drug laws and policies in different countries**

Penalties involving illicit drugs are usually dictated by the social norms of every country or region, and will therefore differ from country to country. In general, most countries will impose lengthy jail terms and/or heavy fines on those caught trafficking or selling illicit drugs. The following is a brief review of some of the differences in drug policies/laws from various countries:

In some countries such as The Philippines, Singapore, China, Saudi Arabia, Malaysia, Thailand, Iran and Vietnam a death sentence is automatically imposed on anyone caught trafficking drugs. In Vietnam and Singapore the possession of even the smallest amount of drugs can lead to a long jail term or even execution. In China and Thailand, rehabilitation in a government facility is mandatory for

possession of drugs [15]. In Singapore, as recently as July 2017, a man was executed for illegal possession of 22.4g of diamorphine [16] despite outcries by the UN and other human rights organisations.

Most European countries prioritise the rehabilitation of those involved in drug trafficking over punishment however tougher legislation against drug trafficking is also the norm across Europe [17].

In the Netherlands, the government approaches the drug problem as a health issue rather than a criminal offence. The country provides more funding towards the treatment of those affected by drug use and in supporting education around drug abuse prevention than it does towards the imprisonment of potential users. Most notably, the Netherlands is the only country to completely decriminalise the use and sale of cannabis [1, 17].

Drug policies in Germany are considered amongst the strictest in Europe, with significant penalties attached to the sale or possession of large quantities of drugs. Notwithstanding this, no criminal action is taken against small-scale possession or the use of many narcotics including cannabis. Rehabilitation of those affected is prioritised; this is witnessed by the provision of supervised “drug rooms” where individuals can safely use their drug of choice and receive counselling when needed [17].

Switzerland has the most liberal policies in the world for drug-related offences. The government put emphasis on “prevention, therapy, harm reduction and prohibition”. The country puts special emphasis towards helping drug addicts receive comprehensive treatment and ensuring the safety of active users. Safe rooms for addicts are also provided [17].

In the United Kingdom, drug related offenses are broken down into three categories by The Misuse of Drugs Act of 1971: Class A, Class B and Class C – with class “A” being the most dangerous (in terms of harmful medical effects) drugs and

class “C” being the least dangerous drugs. Possession with intent to supply carries with it the potential of life imprisonment [2, 17].

Australia advocates for certain harm reduction measures, such as needle exchange programs and also places strong emphasis on drug education in schools at an early age [17]. The laws concerning drug abuse and sale in Australia are similar to those in the United States in that both drug dealers and users are tried in courts of law.

Like most countries around the world, the African continent is also experiencing the challenges of illicit drug use. West Africa has long been associated with the production of cannabis, mainly for local consumption, but it is now also becoming a producer and exporter of synthetic drugs such as amphetamine-type stimulants (ATS). The West Africa Commission on Drugs has declared in their 2014 report that some countries in West Africa are being used as drug transit hubs between South America and the rest of the world. Drug cartels have collaborated with local partners to turn the region into a significant transit route for synthetic drugs produced in South America and Asia to be trafficked into Europe and North America [18].

#### **1.4 Drug laws in the African continent**

Most African countries are member states to the UN and have ratified its treaties and conventions on drug control, therefore legislation on drugs and/or drug-related offences in these countries is influenced by the UN conventions [18, 19]. Categorisation of offences and severity of penalty differ from country to country (in some cases ranging from 10 to 15 years for minor offences and from 15 years to life imprisonment for more serious offences) [20-23]. Certain countries like Botswana and others have inherited rules of procedure and evidence from former colonial powers, such as the English common law rules of admissibility of evidence. Such rules and procedures are however, not always easy to apply especially in certain countries of West Africa [21].

Unlike in Europe and other parts of the world, most African countries have not always prioritised the rehabilitation of end users or put in place measures towards

public education relating to drug abuse. Many have instead always prioritised interventions for the spread and control of HIV/AIDS and malaria or other epidemics over the control of illicit drugs [22-24]. This lack of attention has been used by drug traffickers and a worsening of the illicit drug abuse situation in some countries is evident [24-26].

An analysis in 2009 of grants from the Global Fund to Fight AIDS, Tuberculosis and Malaria – a major source of external funding for West Africa and the world's leading donor for harm reduction – found that Nigeria was the only West African country to include programmes for people who inject drugs in their grant-funded programmes [26]. In most African countries, treatment services are provided by psychiatric hospitals which are generally poorly funded, have inadequate numbers of personnel with no skills and experience in managing substance use disorders. This situation exists in part due to a glaring absence of drug treatment policies, standards and monitoring systems. It is also due to the fact that people who use drugs are often heavily stigmatized, and are deemed as not meriting the expenditure of state resources [26].

Progress towards rehabilitation has however, been made especially after the adoption of the African Union Plan of Action on Drug Control in January 2013. The plan ran from 2013 to 2017 and encouraged member states to ensure that their policies reflect the importance of human rights and public health in drug control [27]. A good example has been observed in West Africa where the West African Commission on Drugs is working to mobilise public awareness and political commitment, develop local and regional capacities and ownership and produce evidence-based policy recommendations focusing on the security and governance impacts of drug trafficking as well as prevention and treatment of drug dependency [27].



### 1.5 South African legislation on drugs

South Africa is made up of nine different provinces (figure 1.1) and has land borders with Namibia, Botswana, Lesotho, Swaziland, Mozambique and Zimbabwe.

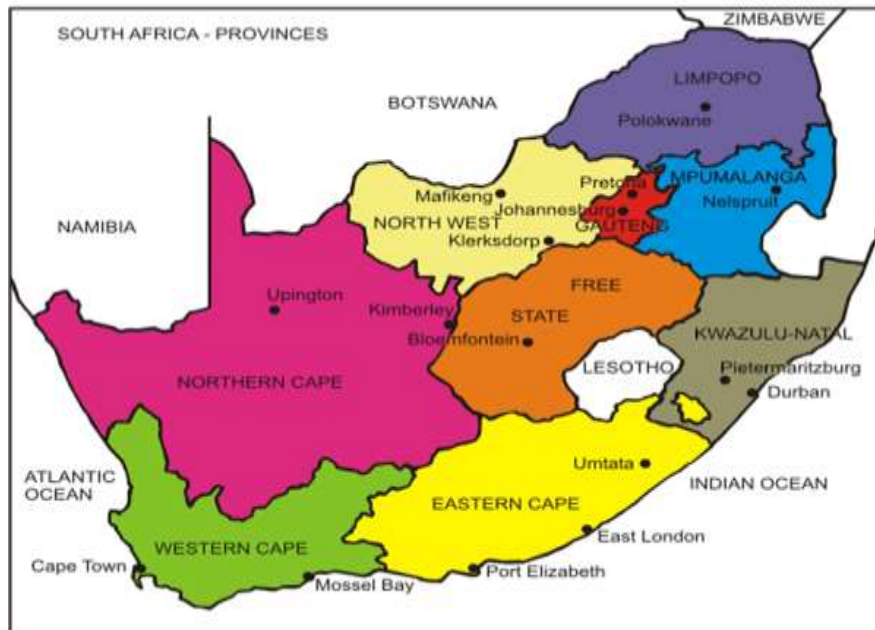


Figure 1.1 Map of South Africa showing different provinces [28]

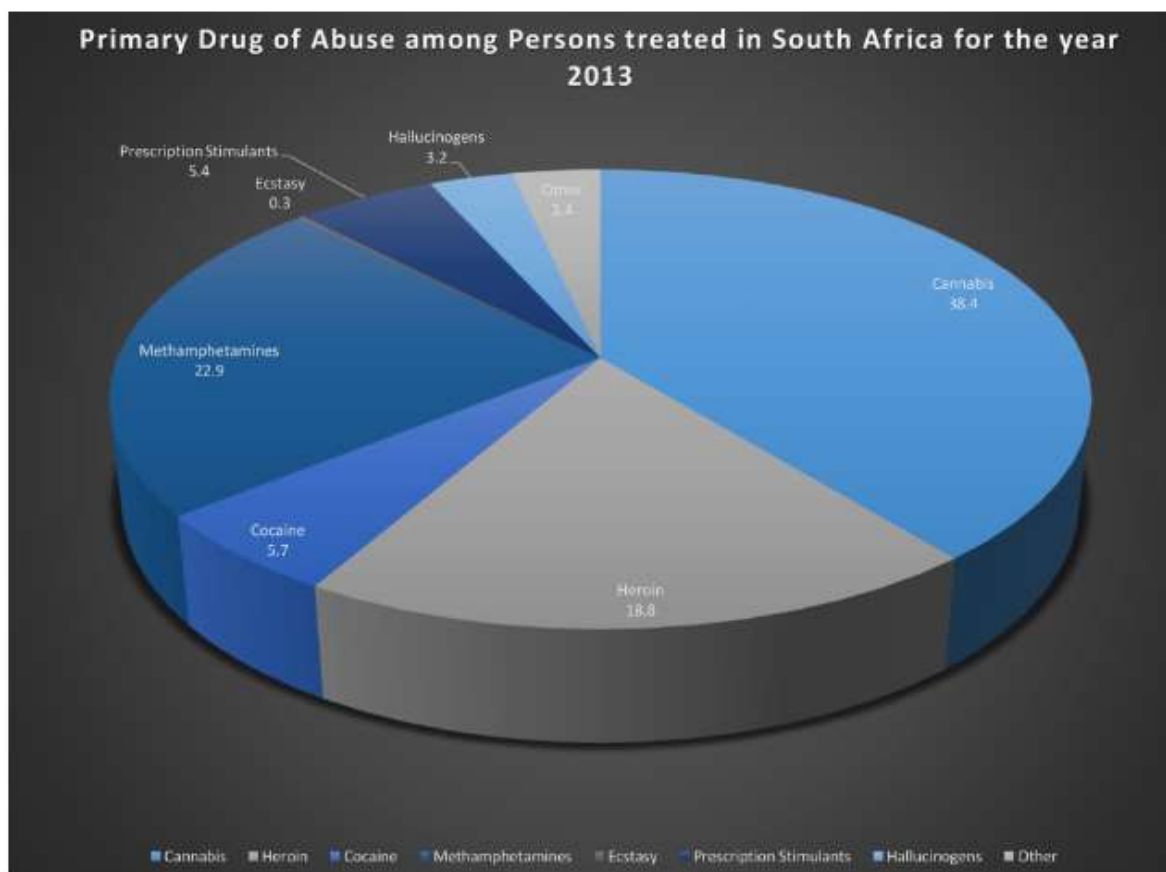
South African law criminalizes three categories of what are known as “dependence-producing drugs” and these are: dependence-producing substances, dangerous dependence-producing substances, and undesirable dependence-producing substances [29]. Offenses relating to “dangerous drugs” (including fentanyl and methadone) and “undesirable drugs” (including cannabis, known as *dagga*, and heroin) are subject to harsh penalties depending on the form of the offense involved. Dealing in any such substances is, on conviction, punishable by a fine and/or a prison term of up to twenty-five years. Use or possession is, on conviction, subject to a fine and/or imprisonment not exceeding fifteen years. Some exceptions apply in both instances [29]

### **1.5.1 Availability and Diversity of Illicit Drugs in South Africa**

In 2015, the World Health Organization identified South Africa as one of the drug using capitals of the world and also that 15 percent of South Africans had a substance addiction problem [30]. Eleven percent of the South African population has also been estimated to experience an addiction disorder during their lifetime [30]. The escalation of drug related crimes in South Africa has allegedly been attributed to the fact that the country has a sophisticated and diversified economy which includes a first world infrastructure which exists alongside widespread and severe poverty [31]. As a result, those in poverty often either sell or use drugs as a coping mechanism. Also when compared to other countries in the continent, South Africa is relatively affluent and diverse and therefore offers the most attractive consumer market for illicit drugs in the sub-continent [30, 31].

The South African government does not publicise any information about the extent to which its people have been affected, therefore available data is only obtained from privately owned drug treatment centres, the media and non-governmental organisations.

Available information on the nation's drug abuse problem (2013) [30] shows that cannabis was at the top of the list, followed by methamphetamine, and heroin as third most abused; that information is summarised in figure 1.2.



**Figure 1.2 Drug abuse numbers from South African treatment centres in 2013 [30]**

In 2016, cannabis was the most abused drug in South Africa; with 60% of substance abuse cases reported. Other drugs mostly encountered were codeine, cocaine, Benzodiazepines, methamphetamine type stimulants, opiates, hallucinogens and methcathinone. Scanty reports on solvents abuse such as glue, petrol and others also exist [34].

### **1.5.2 Nyaope, the new South African street drug**

In addition to experiencing an increase in the prevalence of the above mentioned drugs, South Africa is also facing an overwhelming increase in the abuse of an adulterated new street drug called 'nyaope'. It is alleged to be highly addictive and composed of a cocktail of illicit drugs including cannabis [34-35, 38-45]. There are no formal studies about nyaope and most of available information is obtained from

the media, awareness campaigns, workshops or other materials (figure 1.3) from non-governmental organisations (NGOs) [32].



Figure 1.3 An example of a nyaope flyer from a private rehabilitation centre [33]

According to media reports, nyaope first appeared in the townships of Durban in the early 2000's and has since spread to other parts of South Africa and to some neighbouring countries [39 -41]. It is reportedly prevalent amongst the youth and in high demand, which has since turned it into a multimillion-rand business, mostly run by Nigerian drug lords [41]. Even though the drug is relatively new, a lot of youth and poor people have reportedly become addicted over the years.

Nyaope was eventually criminalised by an amendment of anti-drug laws in March 2014, even though there are still insufficient formal studies about its addiction and its identification in other illicit drug mixtures [46-47]. Under the new law, nyaope users and traffickers are now punished in the same manner as any other traffickers namely, through direct imprisonment [42, 43]. Many people however think that the situation was left unattended for too long and do not think the new legislation will bring things under control [41, 45].

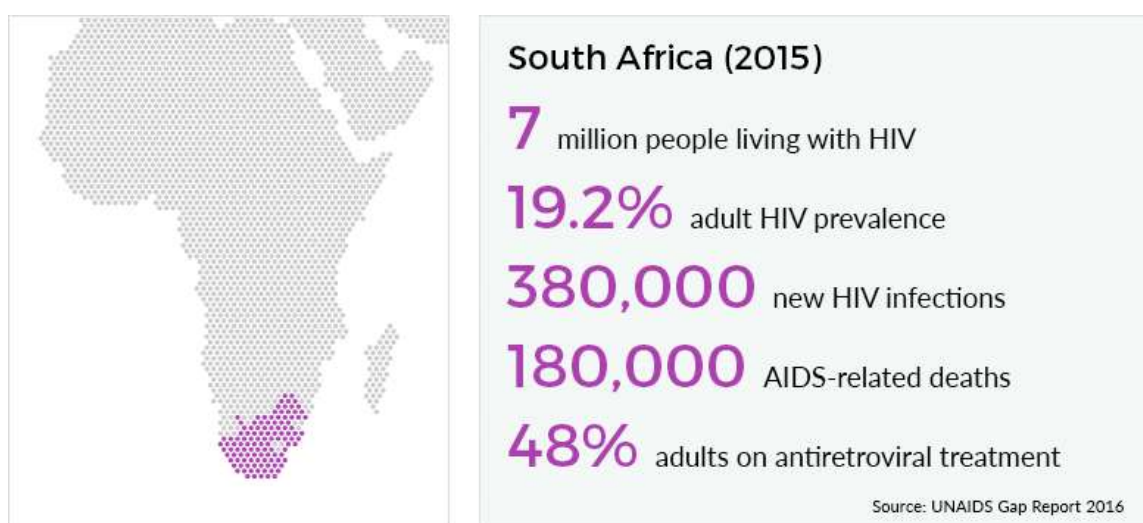
Since nyaope is a relatively new drug combination, its ingredients vary but some reports say it is a cocktail of many illegal substances ranging from heroin, cannabis, methamphetamine and cocaine to pain killers where in each case the material also includes the anti-retroviral drug efavirenz. Efavirenz is believed to prolong the effects of other narcotic drugs in the nyaope mixture [38-45]. Originally nyaope was mixed with cannabis for smoking but nowadays some people choose to chew, snort, inject or heat it up and inhale the fumes [44].

Common names for nyaope differ from province to province; for example, it is known as 'sugars' in KwaZulu-Natal, 'Ungah' in the Western Cape, 'Pinch' in Limpopo and Mpumalanga and 'Kataza' in Johannesburg [49]

Nyaope is the cheapest illicit drug in South Africa; one joint sells for around R30 (£2) [45]. This makes it very affordable and may contribute to its high prevalence amongst the youth. Users are attracted by the initial 'rush and euphoric' effects caused by the drug. These effects however reportedly do not last for long as and are quickly replaced by feelings of drowsiness and deep relaxation. Drug dependency has been reported by the majority of users, who in most cases struggle to get help with their addiction [44-45, 48-50]. Very few drug rehabilitation facilities exist in South Africa and most are privately owned. As a result most people do not get any assistance since they cannot afford the cost or when they do, they soon find themselves in relapse due to lack of availability of proper after-care programmes [48-51].

### 1.5.3 The relationship between nyaope prevalence and the South African anti-retroviral treatment programme

South Africa has the biggest and most high profile HIV epidemic in the world, with an estimated 7 million people living with HIV in 2015. In the same year, about 380,000 new infections were recorded while 180,000 deaths that occurred were attributed to AIDS-related illnesses (figure 1.4) [48].



**Figure 1.4 South African HIV figures as recorded in 2015 [48]**

The South African anti-retroviral treatment is one of the largest anti-retroviral treatment (ART) programmes in the world with all efforts largely financed from its own domestic resources. More than \$1.5 billion is invested annually to run the country's HIV and AIDS programmes [46-50]

Efavirenz is used as part of the HIV/AIDS management programme in South Africa [47-49]. It is prescribed free of charge to patients but due to extreme poverty, some patients are forced to sell their medication to the nyaope industry [38, 41]. One of the new strategic aims adopted by the UNAIDS board is to end the AIDS epidemic as a public health threat by 2030 [50], but this is not going to be possible in South Africa if the nyaope situation is not resolved. So there is a need to both support and address the drug using communities as well as devising new analytical

methodologies for the identification of nyaope and its residues in drug use paraphernalia

#### **1.5.4 People who inject drugs (PWID) and HIV in South Africa**

Data on HIV prevalence among people who inject drugs (sometimes referred to as PWID) in South Africa is very limited and, where it does exist, is based on small sample sizes [49]. According to a study carried out in 2015, an estimated 19.4% of people who inject drugs in South Africa were living with HIV. The study also reported that people who injected drugs accounted for a comparatively low 1.3% of new HIV infections. It was also revealed that 32% of men and 26% of women regularly shared syringes and other injecting equipment and nearly half reused needles [51].

People who inject drugs are also associated with other high-risk behaviours such as sex work and unsafe sexual practices. For example, the above study reported fewer than half of those surveyed used a condom during their last sexual encounter [51]. One implication for this is that the number of people who inject drugs will continue to grow thereby increasing the chances of having new HIV infections; the cycle will continue to repeat itself over and over.

Recently (February of 2017) [52-54], both the South African people and media were shocked by a wave of reports showing injected drug users sharing drugs through a new drug trend called 'blue-tooth'. In these videos and reports, drug users are shown injecting nyaope, and then immediately drawing blood back into the syringe to be injected into a fellow user. This has since raised health concerns for the government, Non-Governmental Organizations as well as rehabilitation centers [53, 54]. Again only the media seems to be outspoken about this new trend.

The South African National Blood Service (SANBS) has since issued a warning about the serious dangers involved with the practice. The general public has been cautioned about the risks involved like the transmission of blood-borne virus or pathogens through the procedure, particularly in South Africa which has one of the

highest HIV infection rates in the world. People are also warned about the fatal consequences of mixing incompatible blood types which is likely to occur if 'blue tooth' is practised [54].

There are also some NGOs and other prominent members of society who insist that 'blue-tooth' is not widely practiced, and blame the media for being careless in reporting and for starting a drug trend that never truly existed. They believe this has escalated the risk for an already vulnerable group [53].

### 1.6 Background information on efavirenz (EFV)

Efavirenz, ((4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1H-3,1-benzoxazin-2-one; trade names: sustiva, stocrin) (figure 1.5) - is a powerful and specific inhibitor for the HIV-1 reverse enzyme recommended for the treatment of HIV/AIDS infections [56-58]. It works by binding directly to the human immunodeficiency virus type 1 (HIV-1) RT, an RNA-dependent DNA polymerase, blocking further replication [60-62]. EFV is a white to slightly pink crystalline powder with a molecular mass of 315.68 g/mol and practically insoluble in water [58].

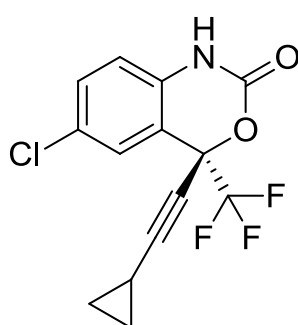


Figure 1.5 Chemical structure formulas for efavirenz

EFV is a weakly acidic molecule, which means it will remain unionised in the acidic environment of the stomach. Thus, theoretically, it is expected to be absorbed primarily in the stomach, where the pH is low. However, some studies have shown that peak plasma concentrations for EFV are reached after 5 hours [59], meaning



that the majority of its absorption takes place in the small intestines. Weakly acidic drugs will still be absorbed in the small intestines even though the pH is higher; this is because of the much longer transit time and the larger absorption surface area [59]. EFV has been viewed as the drug of choice for the treatment of HIV/AIDS infections due to its effectiveness. When combined with other antiretroviral drugs, it has been shown to significantly reduce HIV viral load, which helps prevent damage to the immune system and reduces the risk of developing AIDS [60, 61]. However, EFV has been acknowledged to be psychedelic and that almost half of patients will experience at least one neuropsychiatric side effect at the beginning of treatment. These include nightmares, depression, anxiety, sleep disturbances, impaired concentration, aggressive behaviour, hallucinations, paranoia, and psychosis [62-66].

### 1.6.1 Prescription EFV

Information from literature shows that EFV is prescribed either as tablets or a liquid (figure 1.6) and should only be taken orally [58]. No alternative methods of taking the drug were suggested. Also, known EFV side effects were reported by patients who had been taking the medication orally; it is not known whether the same side effects would be experienced if patients were to take their medication in ways similar to nyaope users. No literature or scientific evidence exists to support or refute the claim that EFV prolongs the effects of other drugs if taken as a mixture (like nyaope). The investigation of the abuse potential of EFV is of interest for this research.



Figure 1.6 Different types of EFV tablets/capsule [58]

A few studies have been performed to investigate the undesirable side effects produced by injecting EFV into healthy laboratory mice. One such study, aimed at investigating the effects of acute and sub-chronic (2 weeks) EFV administration in a series of behavioral tests for anxiety-like and depression-like behavior in healthy rats, confirmed that acute treatment with EFV led to anxiety-like behavior, while sub-chronic treatment induced both anxiety-like and depressive-like behavior [67]. Another study confirmed that the behavioural pharmacology produced by EFV in mice was very similar to that produced by Lysergic acids (LSDs), this was deemed consistent with hallucinogen-type abuse potential and correlates with some of its medication-induced side effects described as adverse neuropsychiatric events [68].

No study however exists to show the behavioural changes produced by EFV or its drug mixture when smoked (i.e. as in the case of nyaope).

To date there is no published work investigating the chemical analysis of samples of EFV which have been heated with other illicit drugs within the nyaope type mixtures. The analysis of the thermal decomposition products of EFV can be used as a potential mechanism for confirming this and therefore elucidate its use in the nyaope mixture and as such has a critical importance within.

## **1.7 Other alleged ingredients of the nyaope drug mixture**

### **1.7.1 Opium and Opiates**

Opium is the sticky or dried latex obtained from unripe poppy seed pod of plant species *Papaver somniferum* L. [69, 70]. Raw opium is a non-homogeneous material often containing poppy capsule fragments. It is sticky, tar-like and dark brown when fresh and is air dried to produce the opium latex, which becomes brittle and hard as it ages. Opium may be cut into small blocks or any other shape and can be taken orally through chewing or inhalation [69, 70]. Opium can also undergo rudimentary refinement to produce a sticky dark product obtained after various treatments of raw opium such as water extraction, in order to make it suitable for smoking. It can

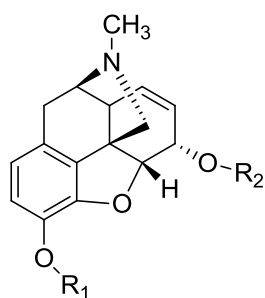
be presented in the form of opium sticks for smoking or in any other shape for inhalation [70].

A number of psychoactive substances (opiates) can be chemically extracted from opium including morphine, codeine, thebaine, papaverine and noscapine. Table 1.1 presents typical percentages concentrations of each opiate present in opium although this can be highly variable.

**Table 1.1 Five main alkaloids of opium [70]**

Major alkaloids	Percentage concentration
Morphine	3.1-19.2
Codeine	0.7-6.6
Thebaine	0.6-10.6
Papaverine	≤0.1-9.0
Noscapine	1.4-15.8

Opiates are naturally occurring alkaloids of the opium poppy (*Papaver somniferum* L.) and can be used both medically and illicitly because of their analgesic characteristics [69]. They have a general chemical structure (figures 1.7 and 1.8), where R represents either –CH<sub>3</sub>, –H or –COCH<sub>3</sub>

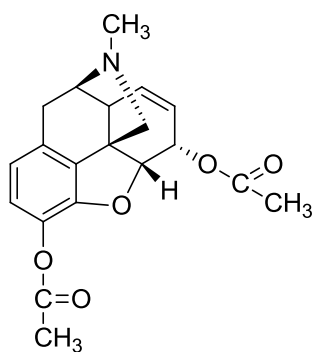


**Figure 1.7 General chemical structure for opiates**

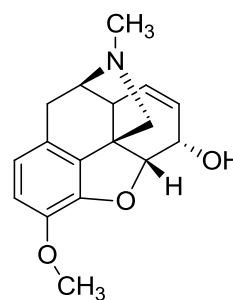
Morphine  $R_1=R_2= H$

Diacetyl morphine  $R_1=R_2= COCH_3$

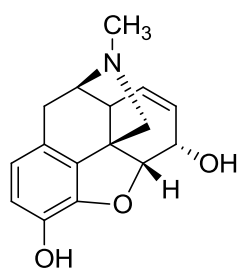
Codeine  $R_1= CH_3, R_2= H$



(a) Diacetylmorphine



(b) Morphine



(c) Codeine

**Figure 1.8 Structural relationships between heroin, morphine and codeine**

Morphine can exist as a fine white powder which is often compressed into blocks with a variety of trademarks or names. Its colour ranges from off-white to dark

brown. It can also be presented in tablet form. The powder form is mainly injected while tablets are taken orally [70].

Diacetylmorphine (sometimes called diamorphine) is a semi-synthetic opiate synthesized from morphine. There are two main formulations; the water soluble diacetylmorphine hydrochloride salt and the relatively water insoluble diacetylmorphine base. Diacetylmorphine, when mixed and diluted with other compounds is known as heroin, (in some parts of the world, heroin is also the term used for diacetylmorphine) and can appear in a range of colours from white to brown [70]. Heroin production is generally limited to only four regions of the world namely South-West Asia, South-East Asia, Central America and South America; as such there are a variety of names that are used to designate the heroin in its various stages of refinement/purification which depend on the complexity of the processes used. These can include, for example, brown heroin and black tar heroin. Heroin is mainly taken by injection, nasal insufflation or smoking. Following injection, diacetylmorphine is rapidly broken down in the blood to the pharmacologically active 6-monoacetylmorphine (6-MAM) and then to morphine, the major active metabolite [70].

Compared to morphine, diacetylmorphine is more fat-soluble (due to its two acetyl groups), and crosses the blood-brain barrier more rapidly, reportedly in 15-20 seconds, and achieves relatively higher levels in the brain following intravenous injection, with almost 70 per cent of the dose absorbed into the brain. Following oral administration of diacetylmorphine undergoes extensive breakdown to morphine [69, 70].

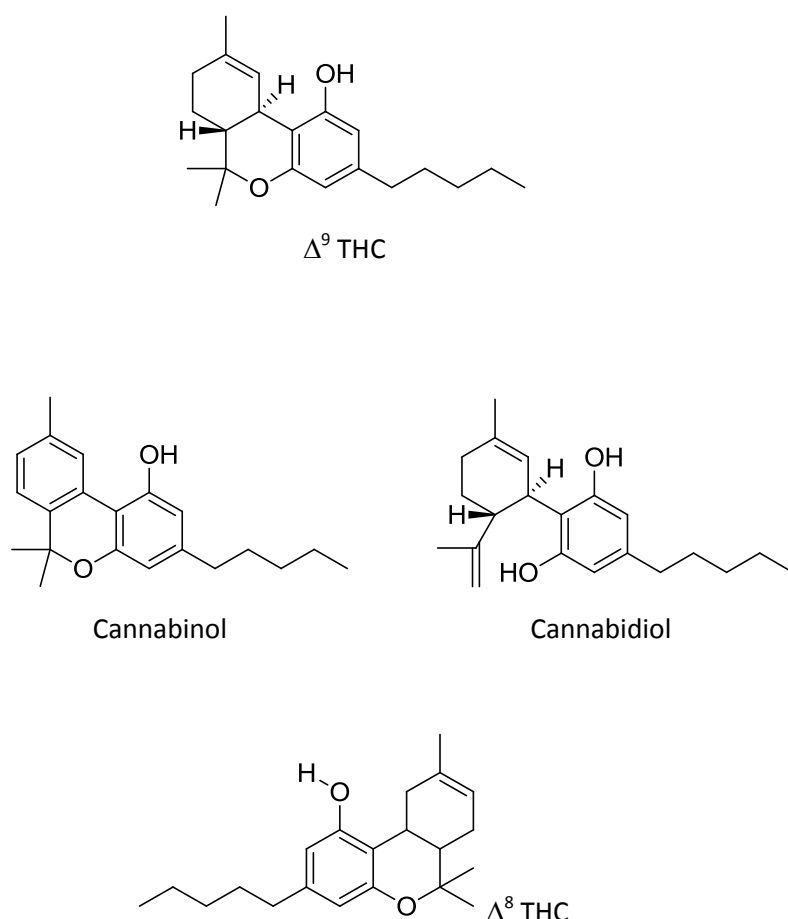
The pharmacological effects of morphine, diacetylmorphine and other opiates are mediated through their interaction with opioid receptors and inhibitory neurotransmitters (table 1.2). Opioid receptors are responsible for triggering brain reward systems and producing analgesia (pain relief) by decreasing pain transmission. Different types of opioid receptors exist; among them are mu ( $\mu$ ) receptors, which mediate analgesic and behavioural effects [70].

**Table 1.2 Pharmacological effects of opiates [70]**

<b>Desired effects</b>	<b>Severe effects</b>	<b>Effects due to long term abuse</b>
Euphoria	Drowsiness	Rapid development of tolerance, physical and psychological dependence
Contentment	Potential nausea and vomiting	Malnutrition
Detachment from emotional and physical stress	Potential stimulatory effects	Chronic sedation and apathy
Analgesia	Constriction of pupils	Respiratory problems if smoked
	Possible respiratory depression leading to death	Irregular menstrual periods
		Withdrawal syndrome
		Damage to nose due to snorting or sniffing

### **1.7.2 Cannabis Products**

Cannabis products are produced from the *Cannabis sativa L* plant [70], which exists in many different biological, chemical and morphological varieties; a “dioecious” species in which the plant can be staminate (male) or pistillate (female), but also the “monoecious” or hermaphrodites, where both sexes coexist on one plant [3]. The major psychoactive substance of the *Cannabis sativa L*. is (-)-trans-delta- 9-tetrahydrocannabinol (delta-9-THC or THC), which has some hallucinogenic effects. Other minor psychoactive substances like cannabidiol, cannabinol and delta-8-THC also exist [70, 71]. The chemical structures are presented in figure 1.9.



**Figure 1.9 Chemical structures of the four main cannabinoids**

#### **1.7.2.1 Cannabis herb**

Cannabis (herb), also known as grass or weed, is a green or brown dried material generally made from the dried leaves and flowering parts of the female plant and looks like tightly packed dried herbs (figure 1.10) listed in Schedule I and IV of the Single Convention on Narcotic Drugs of 1961 [13]. A commonly encountered form is the loose material (in a small roll wrapped in paper or onto sticks) and administered by inhalation (smoking, vaporization). There is also ground material which is melted in butter to produce “cannabutter” or “hash brownies”, or “space-cake” for oral consumption. The powder can also be infused with hot water as a drink [70]



**Figure 1.10 Cannabis herbs [72]**

Skunk' (figure 1.11) is comprised of a range of stronger types of cannabis, grown for their higher concentration of tetrahydrocannabinol (THC). It can be grown either under grow-lights or in a greenhouse, often using hydroponic techniques. It has been reported to have THC content 2-3 times higher than the cannabis herb [73].



**Figure 1.11 Skunk cannabis [74]**



It has also been reported to work much quicker, and capable of producing hallucinations with profound relaxation and elation – along with nervousness, anxiety attacks, projectile vomiting and a strong desire to eat [73]. Skunk has reportedly ‘invaded’ the UK cannabis street market over the last 15 years. This is mainly attributed to the fact that people are allowed to buy seeds over the internet [73].

#### **1.7.2.2 Cannabis resin**

Cannabis resin (figure 1.12) is a dried brown or black resinous secretion obtained from the flowering tops of the cannabis plant which can be categorised further as crude or purified depending on its quality and consistency [70]. Cannabis resin is placed under Schedule I and IV of the Single Convention on Narcotic Drugs of 1961 [15]. The resin may be encountered as fine powder compressed into slabs or as loose or pressed sticky powder or pressed into slabs, rods, or other shapes. It may be inhaled, taken within food or as tea [70]



**Figure 1.12 Cannabis resin [70]**

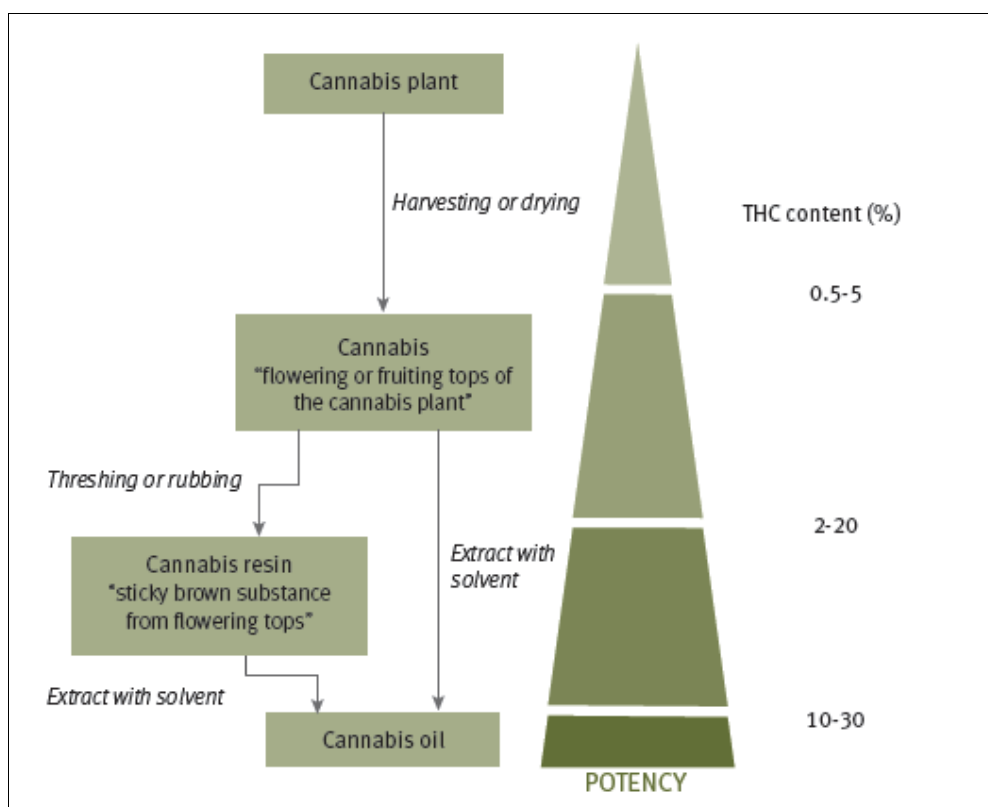
### 1.7.2.3 Cannabis (hash) oil

Cannabis oil (figure 1.13) is obtained by extraction of the crude plant material, or cannabis resin with an organic solvent. The extract is then filtered and evaporated to give oil. It is tar-like reddish to brown or green viscous liquid which is normally mixed with tobacco for smoking, used within a vaporiser or taken orally [70].



Figure 1.13 Cannabis oil and resin [70]

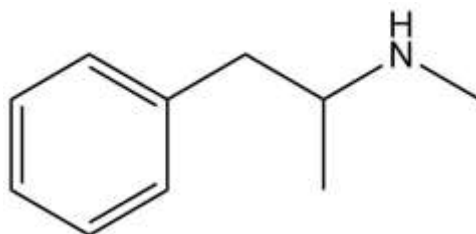
The potency of the various manifestations of cannabis depends very much on the concentration of the active ingredient (delta-9-THC) which varies depending on formulation and refinement. Moving from plant to resin to oil concentrates the active ingredient as presented in figure 1.14.



**Figure 1.14 Production of various cannabis products and their potency [70]**

## 1.8 Methamphetamine

Methamphetamine (figure 1.15) found in illicit markets is predominantly produced in clandestine laboratories [70]. This is mostly related to the simplicity of its manufacture and the availability of a variety of precursor chemicals and methods of synthesis; some of which are readily available on the internet.



**Figure 1.15 Chemical structures for methamphetamine**

Methamphetamine is synthesised through various synthetic routes. Two major groups of synthesis can be distinguished: (a) syntheses starting from 1-phenyl-2-propanone (P-2-P) and yielding racemic methamphetamine, such as the Leuckart route and reductive amination; and (b) synthesis routes using optically pure *l*-ephedrine or *d*-pseudoephedrine as starting materials, thus yielding the more potent *d*-methamphetamine. The latter include the Nagai route, Birch reduction, Rosenmund hydrogenation, and the Emde route with chloroephedrine as intermediate [70, 76]

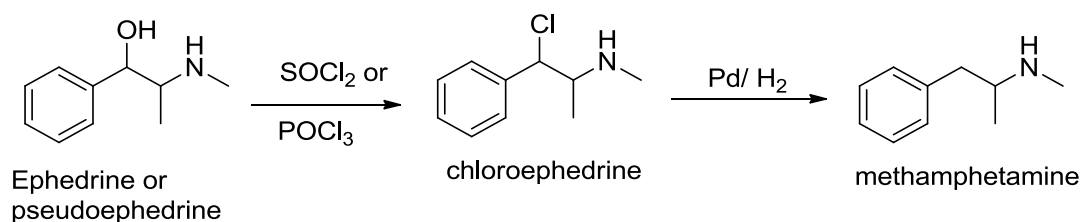
### **1.8.1 Ephedrine based precursor methamphetamine**

Methamphetamine synthesised in clandestine laboratories using ephedrine compounds is mostly through the Emde [70, 77] (figure 1.16) and Nagai routes [79-89] (figure 1.17). The Birch reduction (figure 1.18) method is not popular due to potential hazards involved [70, 78].

Ephedrine synthetic methods involve non-metal reductions (Nagai), dissolving metal reductions (Birch) and heterogeneous catalytic reductions (Emde) reactions [79, 80].

#### **1.8.1.1 The Emde synthetic route**

This method proceeds through the reaction of ephedrine or pseudoephedrine with thionyl chloride to give the intermediate chloroephedrine, which is then hydrogenated over a platinum or palladium catalyst to yield methamphetamine. The chloroephedrine intermediate is rarely found as an impurity when using gas chromatography analysis because it decomposes to form aziridines. It also decomposes rapidly during basic extraction of methamphetamine [70, 82-85].

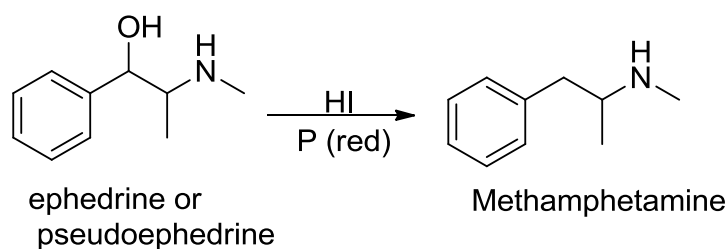


**Figure1.16 Methamphetamine synthesis via the Emde synthetic route**

#### 1.8.1.2 The Nagai synthetic route

The synthesis starts by heating ephedrine or pseudoephedrine with red phosphorus and hydriodic acid. The reaction mixture is then filtered, basified and extracted into a solvent. Methamphetamine base is formed. It is an oily liquid, commonly referred to as “meth oil”. This is followed by crystallisation of hydrochloride salt using ether/acetone and hydrochloric acid. Alternatively, hydrogen chloride gas is bubbled through the meth oil causing the hydrochloride salt to precipitate out of the solution [70, 80].

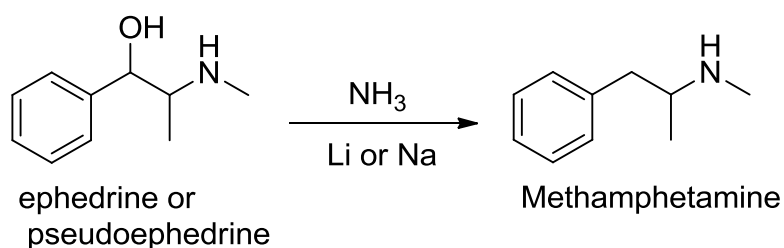
HI and red phosphorus can be replaced by iodine and hypophosphoric acid (sodium hypophosphate), or by water and iodine. In rare occasions, the reaction mixture, sometimes known as “ox-blood”, is used without further purification, mostly by injection. The red coloured mixture, caused by an excess of iodine, contains “meth oil” and different impurities related to the HI/red P route [79, 80]. Typical impurities found in samples produced by reductions involving HI/red P or iodine/hypophosphoric acid are ephedrine or Pseudoephedrine, aziridines and dimethylnaphthalenes [77-79, 85]. Aziridines cannot be considered as route-specific impurities since they can be also produced from chloroephedrine by halogen elimination and ring closure (Emde method), or from an oxime intermediate and N-hydroxymethamphetamine [77, 78, 85].



**Figure 1.17 Methamphetamine synthesis through the Nagai synthetic route**

### 1.8.1.3 Birch reduction synthetic route

The process involves mixing ephedrine or pseudoephedrine with anhydrous ammonia gas and either sodium or lithium metal. The mixture is allowed to stand until the ammonia has evaporated. Isolation of the meth oil is carried out by direct solvent extraction and filtration. The product is further purified by formation of the hydrochloride salt and re-crystallisation. In illicit practice, Birch reduction is usually completed in a one-step reaction using widely available ammonia, and lithium strips from batteries [70]. Several route-specific impurities such as N-methyl-1-(1-(1, 4-cyclohexadienyl))-2-propanamine are reportedly produced through this route. Reactions involving anhydrous ammonia have been associated with clandestine laboratory explosions [70, 81].



**Figure 1.18 Methamphetamine synthesis via the Birch synthetic route**

### 1.8.2 Methamphetamine synthesis using Phenyl- 2-propanone (P2P)

The most popular synthetic route employed for the illicit manufacture of methamphetamine is the Leuckart reaction method (figure 1.19) [70, 75]. It involves a non-metal reduction reaction usually carried in three steps. First a mixture of P-2-P and methyl formamide (in the presence of formic acid) is heated until a condensation reaction results in the production of an intermediate product N-formylmethamphetamine. In the second step, N-formylmethamphetamine is hydrolysed typically using hydrochloric acid. The reaction mixture is then basified, isolated, and (steam) distilled. In the final step, the product is precipitated out of the solution, typically as the sulphate salt [70, 76].

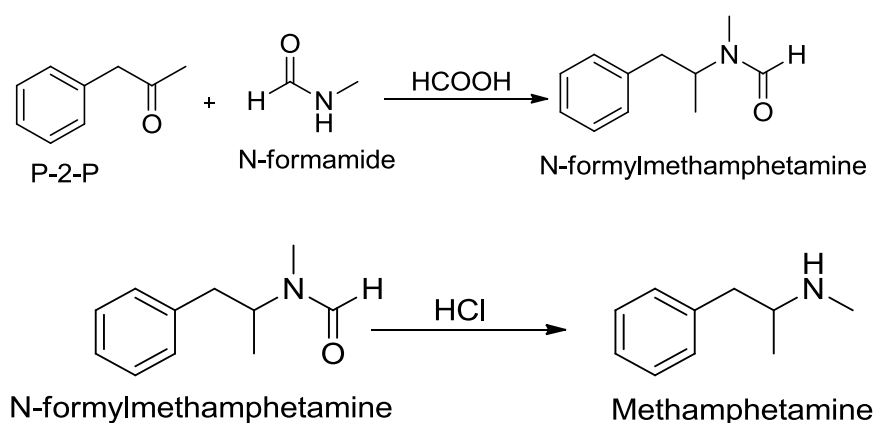
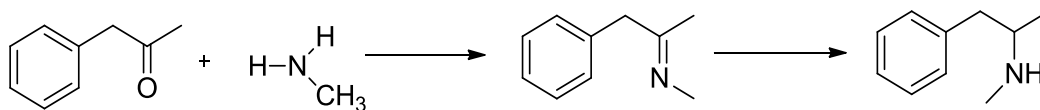


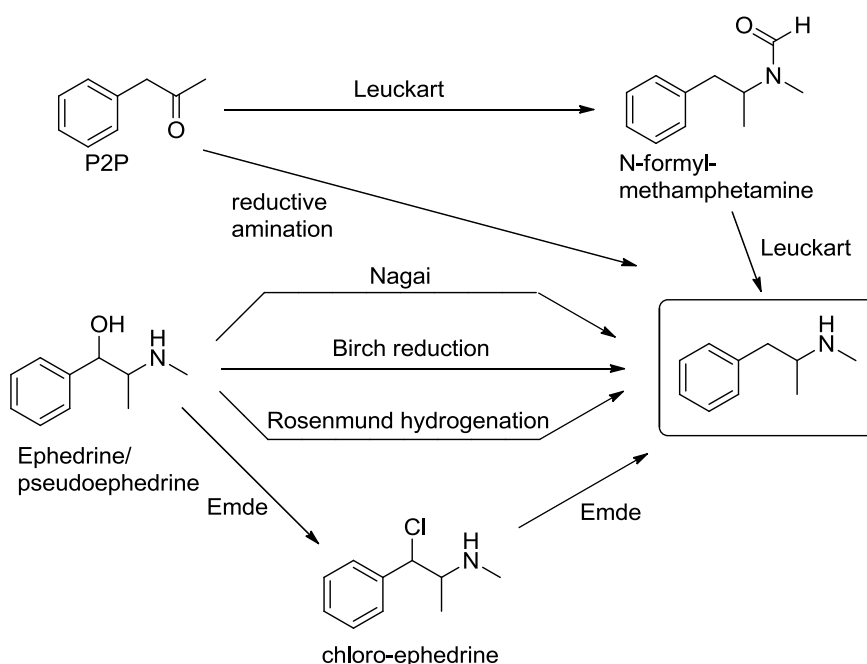
Figure 1.19 Methamphetamine synthesis via the Leuckart synthetic route

The second common methamphetamine synthesis method involves a reductive alkylation reaction of an aldehyde or ketone with a primary or secondary amine in the presence of hydrogen and a hydrogenation catalyst (figure 1.20). The reductive alkylation of P2P and methylamine with hydrogen and Pd/C (palladium on carbon) produces methamphetamine. The reductive alkylation of P2P and IV-benzylmethylamine produces N-benzylmethamphetamine-also known as benzphetamine. Subsequent hydrogenolysis of benzphetamine produces methamphetamine [9, 70, 77-80].



**Figure 1.20 Methamphetamine synthesis via the reductive alkylation of phenyl-2-propanone with methylamine**

Commonly encountered methamphetamine forms are the white to light brown powder for nasal insufflation or smoking, the injected solution form (drug powder dissolved in saline or distilled water), tablets and capsules (in different shapes and colours) for oral consumption and crystalline methamphetamine for nasal insufflation or smoking [70]. The different methods through which these forms are administered impacts the onset and duration of action; for example, insufflation causes quick absorption into the bloodstream through the mucosa and so enables a more rapid onset of effects than oral consumption. In general, only methamphetamine is commonly found in the crystal form. Methamphetamine is placed under Schedule II of the Convention on Psychotropic Substances of 1971 [14]. The common methods for synthesis described are summarised in figure 1.21.



**Figure 1.21 Common synthetic methods for methamphetamine**



### **1.9 Analytical challenges of the nyaope street drug**

A lot of qualitative research has been conducted on the nyaope street drug [31-47], from which information regarding the various ingredients used to make the mixture is now available. It has been revealed that the drug can contain a mixture of illicit substances including opium, heroin, cannabis, some amphetamine type stimulant drugs as well as the anti-retro viral drug, efavirenz, as the main ingredient. Even though this information is available, no laboratory studies have been conducted to confirm the alleged components of the nyaope.

Analytical methods for the identification and quantification of pyrolysis products of most illicit drugs which are alleged to be components of nyaope street drug have already been developed [73, 81-85]; however none of these includes the identification of efavirenz or its pyrolysis products. An analytical strategy for the identification of alleged components of the efavirenz in both powder and smoked products was therefore necessary. Gas Chromatography Mass spectroscopy (GCMS) is the most common technique used by forensic laboratories to analyse of illicit substances [86, 87]. It is a very discriminatory technique and does not require a lot of sample. Volatile pyrolysis products of efavirenz can be easily analysed with the GCMS technique while non-volatile products can be identified with High Performance Liquid Chromatography (HPLC) technique coupled with Mass Chromatography or other techniques such as Ultra Violet-Visible Spectroscopy (UV-Vis) or Infra-Red Spectroscopy (IR). The two techniques are also common in forensic analysis of drugs [83, 86] Other techniques which can be applied for the analysis of nyaope samples and its smoked products include Fourier Transform Infrared Spectroscopy (FTIR) for elemental additives such as calcium and magnesium and Time of Flight Mass Spectroscopy (TOF-MS), capable of detecting illicit substances and other substances present in minute quantities in the nyaope mixture. The last two techniques are however not commonly encountered in most forensic laboratories in Africa and were not considered for this research.

### **1.10 Objectives of the study**

The main objective of the study was to develop and validate an analysis method for the pyrolysis products of efavirenz within common drug mixtures (heroin, opium, cannabis, methamphetamine, tobacco) using both the GCMS and the HPLC.

Other objectives include:

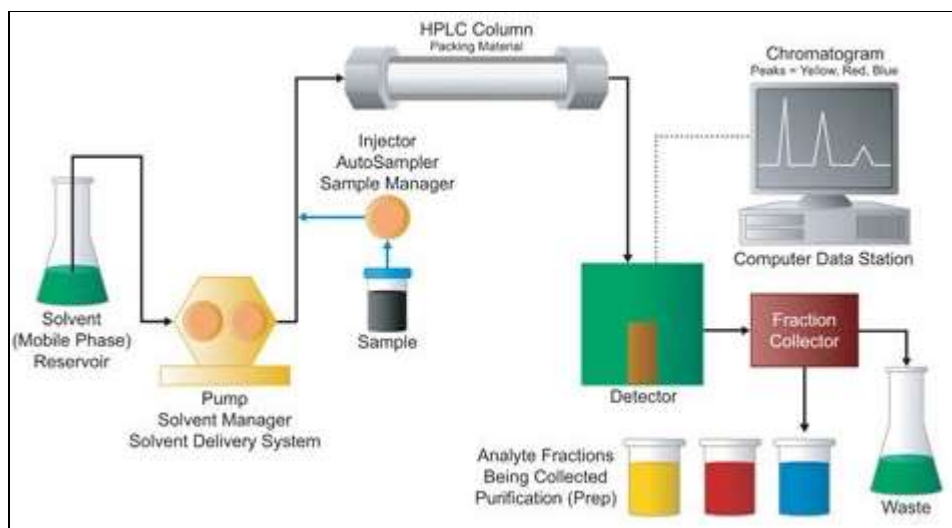
- i. To optimise extraction conditions for activated carbon strips (ACS) as a trapping media.
- ii. To analyse, and where possible identify, the extracted pyrolysis products for each drug using the GCMS and HPLC.
- iii. To analyse, and where possible identify, the extracted pyrolysis products for each drug mixture in the presence of EFV, as simulated nyaope samples, using the GCMS and HPLC.

## **CHAPTER 2: ANALYTICAL TECHNIQUES**

### **2.1 Introduction**

Abuse of certain drugs involves smoking, which results in the generation of thermal decomposition products. These products can be studied to reveal certain information such as the drugs' pyrolysis patterns or their behaviour with change in temperature. The ability to analyse the thermal decomposition products of efavirenz (EFV) may present an objective analytical method for confirming the use of efavirenz in residues of smoked nyaope mixture. Pyrolysis products of most illicit drugs including those from the five alleged components of nyaope drug (cannabis, opium, heroin, amphetamine and methamphetamine) have been successfully studied using both the GCMS and LC coupled with other detectors such as MS, UV-Vis and Diode Array detectors [88]. Information obtained was in some cases used to establish linkages between two or more drug samples, drug toxicity studies, guidance to analytical approach, or determination of methods of drug synthesis as the case with methamphetamines [81-85]. It was therefore proposed that the two analytical techniques of Gas Chromatography-Mass Spectrometry (GCMS) and High Performance Liquid Chromatography coupled with Diode-Array Detector (HPLC-DAD) be used to establish the identity of the various thermal decomposition products resulting from thermal decomposition of 5 well known controlled drugs (cannabis, opium, heroin, amphetamine and methamphetamine), EFV and the combination of each drug with EFV which mimics nyaope samples.

When using the two techniques, volatile organic pyrolysis products of the nyaope can be detected by the GCMS following adsorption and desorption with a suitable solvent after collection of headspace sample with the Activated Carbon Strips (ACS). The HPLC-DAD can be used to detect both volatile (from the ACS) and non-volatile (from the sample matrix) pyrolysis products. When using the HPLC both volatile and non-volatile products can be collected in the mobile phase as they elute from the column, using a fraction collector downstream of the detector flow cell. The process is called preparative chromatography [89] [figure 2.1].



**Figure 2.1 Schematic representation of selective preparative Chromatography [89]**

In preparative chromatography, individual analytes are selected and collected as they elute from the column. The fraction collector selectively collects the eluate that only contains a purified analyte, for a specified length of time. The vessels are moved so that each collects only a single analyte peak [89]

## 2.2 Derivatisation

Drugs are often chemically derivatised before analysis with the GC-MS for the following reasons: (a) to bring the analytes to the chemical forms that are more compatible to the chromatographic environment, (b) to create a separation mechanism or to maximize resolution efficiency, (c) to improve detection or structural elucidation effectiveness or (d) to make use of the analytes' specific structural features for analytical needs [90]. Analytes that are strongly acidic, basic or with functional groups, that may not vaporize or may interact with (irreversibly or reversibly) silanol groups or contaminating compounds present in the chromatographic system, can be more effectively analysed after chemical derivatisation. Enantiomers can be chromatographically resolved by achiral columns after being converted into diastereomers using chiral reagents; derivatisation may also bring the retention time of the targeted analytes to a more desirable range.

Introduction of certain elements or groups through chemical derivatisation may enhance the detector's response or generate mass spectra helpful to the elucidation of the analytes' structural features [91]. Most drugs alleged to be components of nyaope, some of which are used in this study have been derivatised before [92]. However derivatisation may also complicate the interpretation of the analytical data because the resultant reaction may introduce impurities, uncertainty on the completeness of the analytes' conversion, and other interference factors. For this reason and also since it was the first study involving the analysis of EFV with the GCMS, derivatisation was not performed.

The pyrolysis experiment was conducted based on the temperatures obtained from literature on the pyrolysis of a cigarette, where pyrolysis products are mostly generated through mild pyrolysis. Through this type of pyrolysis, products generated are in the form of smoke particles in which pyrolysis products and other compounds are in their gaseous state; as a result these can be concentrated in the sample headspace, trapped with a porous medium and later be extracted with suitable solvents for analysis. The choice of trapping media is not limited; studies involving the use of different trapping media including the use of activated carbon strips have been conducted [93-103]. ACS is a porous mixture of activated charcoal on Teflon bedding [104, 105]. It is normally suspended above the headspace of the material during pyrolysis to adsorb the released gaseous products. The advantage of using ACS is that it is fairly easy to set up, quick, cheap, sensitive, portable, non-destructive [103-106] and does not undergo oxidation. The non-destructive nature of the technique allows for archiving of extracts either as adsorbed strips or as the extraction solution. The strips are also hydrophobic in nature and therefore not affected by moisture. All these advantages make the ACS a suitable trapping for pyrolysis products of nyaope especially in Africa where analysis of samples can take longer than expected due to instrument breakdowns or power outages. It is also common to send samples across borders for analysis or in between laboratories hence the need for robust trapping media like the ACS. However, these have never been used before for the adsorption of pyrolysis products of drug, a validated ACS method was therefore necessary.

### 2.3 Principal of chromatography

Chromatography is an analytical technique commonly applied during the separation of a mixture of chemical substances (in a sample) into its individual components, so that each component can be thoroughly analysed [106]. The process can be both qualitative and quantitative as it allows the analyst to estimate the numbers of components in the mixture and to quantify them as well. The sample gets separated under the influence of a mobile phase over a stationary phase [107, 108]. The separated components can be identified later and quantified. The stationary phase can be either a solid or liquid supported on a solid while the mobile phase can be either a liquid or gas [106, 107]. The mobile phase flows through the stationary phase and carries the components of the mixture along. Different components travel at different rates depending on their affinity to the stationary phase. Components with more affinity to the stationary phase will spend more time in the column than those with less affinity.

Retention time (RT) is a measure of how long each component takes to travel from the instrument sample injection port to the detector. It is characteristic of the identity of the component under the operating conditions [108]. Identity of the component can be confirmed through the analysis of reference material under the same operational conditions. The matching of retention time of reference material and the component peak is used to confirm the identity of the unknown sample component [108]. Detector response for each component is presented in the form of a chromatogram (figure 2.1) [109]. A chromatogram is a two-dimensional plot of analyte concentration versus retention time [108], whose height or area represents the concentration of the particular component.

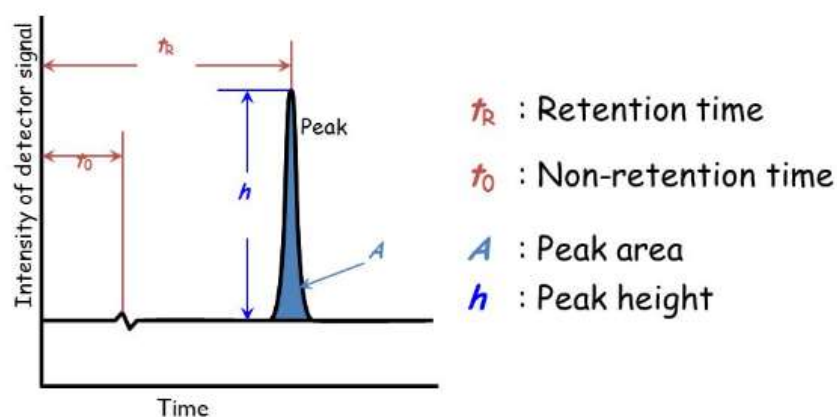


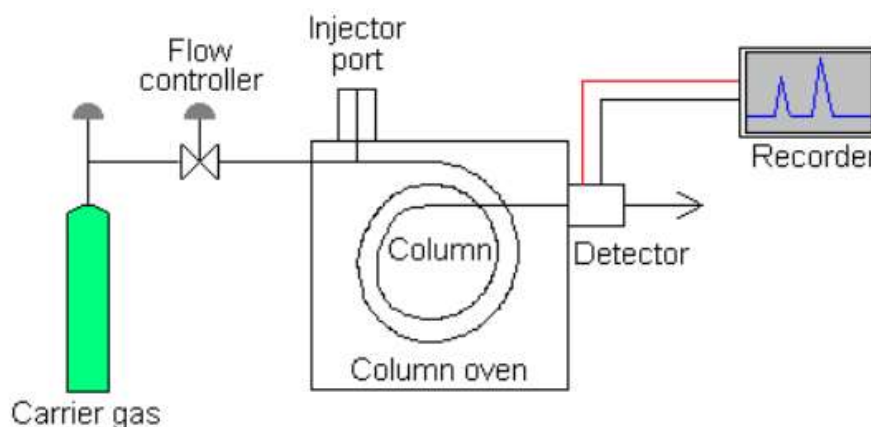
Figure 2.1 Schematic diagram of a GC chromatogram [109]

## 2.4 Gas Chromatography (GC)

Gas chromatography facilitates the analysis of substances within gaseous chemical mixtures by separating the sample into individual components and timing how long each spends interacting with the stationary phase of the analytical column after injection, before reaching the detector where compound identification can occur. Compounds with less affinity to the analytical column will have a shorter retention time compared to those with high affinity for the stationary phase [110].

A mobile phase of (normally) high purity inert carrier gas is used to sweep the vaporised samples out of the injection block of the instrument (where vaporisation occurs) through an analytical column containing the stationary phase to a detector for identification. Helium or hydrogen gases are normally used as carrier gases even though argon, nitrogen and carbon dioxide are also suitable [109-112]. Moisture and gas traps/filter systems are installed along the gas line, a few centimetres before the gas enters the instrument so that contaminants which may damage the analytical column or instrument can be detected. These protect both the instrument and the analyte by removing impurities and moisture before they reach the instrument. Water can affect analytical column polarity and peak shape while the presence of oxygen may affect sensitivity and peak shape [113-115]. Contaminants may affect both instrument sensitivity and analyte purity. The GC in a

simplistic form is comprised of the injection port, column and detectors and is represented in figure 2.2.



**Figure 2.2 Schematic diagram of a GCMS [113]**

The injection port, also known as the sample inlet is where samples (liquid or gas) are introduced into the GC for analysis. Inlet temperatures are usually set higher than the boiling point of the least volatile component in the sample and maintained at high temperature so as to vaporise the sample as soon as it is injected [112].

GC injections are undertaken either in the split or split-less modes [114, 115]. In split injection mode, only a portion of the vaporised sample is transferred onto the head of the column while the remainder of the vaporised sample is removed from the injection port via the split vent line [114, 115]. Split injections are only performed with highly concentrated samples which will still produce and maintain a good detector signal even after a portion of the sample has been discarded during the injection process [114]. On the other hand, a split-less injection is performed when target analyte concentrations are low in the sample such that splitting of the sample would not produce a sufficient signal within the detector [114]. The process of performing either mode of injection is controlled by changing both the flow path and rate of carrier gas through the injection port [114, 116]. In split injections, the flow rate is set high so that the sample is rapidly swept through the injection port liner, past the column and out the split vent [116]. This will result with only a



minimal amount of the sample being directed into the column for analysis. While in split less injections, the flow rate is set relatively slow so that most of the sample is directed into the analytical column [116].

The analytical column is housed inside a temperature controlled oven. There are two types of GC columns, packed and capillary [110, 113-117] columns. Packed columns have a finely packed inert solid supporting material. These columns usually measure between 1.5 and 10 metres with a thickness of 2 to 4 mm and are suitable for the analysis of gas samples [114]. Capillary columns are usually very thin ( $\pm 0.30\text{mm}$  i.d) and can either be wall coated open tubular (WCOT) or support coated open tubular (SCOT) [114]. WCOT columns walls are coated with liquid stationary phase whereas the stationary phase of the SCOT column is lined with a thin layer of solid support on to which liquid phase is adsorbed [113, 114]. SCOT columns have a better separation efficiency compared to WCOT columns because of the increased surface area of the stationary phase coating [113, 114]. Capillary columns are suitable for most analytes including drug screening

#### 2.4.1 Column Efficiency Test

The assessment of column packing efficiency is used to provide valuable information on column performance and deterioration throughout the entire analysis [111, 113]. The efficiency of a column can be determined by the Number of Theoretical Plates present [113-115]. One way of doing so is by applying equation 3.1;

$$N = 5.54 \left( \frac{t}{w_{1/2}} \right)^2$$

Where t is retention time and W is peak width [7]

**Equation 3.1 Calculation of theoretical plate [110]**

Columns with high plate numbers are considered more efficient than those with a lower plate count. However, literature does not give any specific numbers for

reference. High efficiency columns are very desirable because they are thought to produce narrower peaks which are easy to resolve [112, 113, 115]. Chromatography theory also states that doubling the length of a column increases the resolving power only by the square root of 2 – i.e.  $\sim 1.4$  times [89]. The efficiency can be normalized with the length of the column to give the height equivalent theoretical plate, called HETP or H [89]. The Van Deemter equation describes the various factors influencing H, and is divided into eddy diffusion, longitudinal diffusion, and mass transfer terms. The relative importance of these factors varies with mobile phase velocity. Particle size and morphology contribute to H, along with a variety of other factors. Understanding the van Deemter equation allows the determination of the optimum mobile phase velocity [89]. Stationary phase particle size is one of the factors contributing to the efficiency of the column and therefore very important in the Van Deemter equation. For a given column length, the plate number ( $N_{th}$ ) of a column is inversely related to the particle size of the column packing; the smaller the particles, the higher the plate number and the separation power [89]. The plate number is also dependent on the flow rate (F) of the mobile phase. Optimum velocity is the velocity at which the plate number is highest (and H is lowest). A lower or a higher flow rate provides fewer plates (higher H) [89, 90]. The equation describes the various contributions to plate height (H) in three terms:

$$HETP = A + B/u + Cu \quad [90]$$

Where H is equivalent to a theoretical plate height (length of column/number of theoretical plates)

A = Eddy diffusion term and only applies to packed columns

B = longitudinal column length/ordinary molecular diffusion term; this is only observed in tendency of sample peaks to broaden at low flow rate in the column [90]

C = resistance to mass transfer term resulting from pushing a peak through a column at high flow rates or when using thicker films. This limits sample interaction with the stationary phase [90]

Eddy diffusion can be minimised through the use of well packed columns, smaller stationary phase or using particles with a narrow size distribution. Effects of longitudinal diffusion can be minimised by using higher mobile phase flow rates, short and narrower system tubing and the use of correct nuts, ferrules and fittings wherever possible. Mass transfer can be minimised by using smaller (diameter) stationary phase particles, lower mobile phase flow rates and heating the column (at higher temperatures the diffusion processes are speeded up and the differences in elution time from the particle pore are reduced) [90].

There are a range of detector systems available and commonly used with a GC system. Within this research work the detection of the samples was performed using a mass spectrometer.

## **2.5 Mass Spectrometry (MS)**

A mass detector analyses the pattern of molecular ions produced when a molecule is broken down during ionisation to generate a mass spectrum of the compound [113, 115, 116]. Ionisation can be achieved through either the creation of a radical cation (also known as Electron Ionisation, EI) or through charge transfer (Chemical Ionisation, CI) [117, 118]. Charge transfer involves the removal or addition of electrons to molecules or atoms to produce ions [118]. For EI, a sample is ionised by bombarding it with a beam of electrons. This results in the loss of electrons and creation of a molecular ion [112]. When the molecular ion is detected by the mass detector, its representative peak is displayed together with its molecular weight.

Molecular ions are usually the heaviest ions and their mass is usually used to identify the parent compound [112]. Molecular ions are very unstable and often fragment further into smaller ions (called daughter ions) and are sorted within the mass spectrometer according to their mass to charge ratio [112]. The sequence of fragmentation can be very specific to the individual compound and the specificity of the mass spectrum generated upon ionisation of an analyte provides a 'signature' for its molecular structure. This information may then be used to identify the

compounds of interest and help construct the structure of unknown components of a mixture [112, 113].

For chemical ionisation, methane or another suitable gas is first ionised to create a free radical which in turn can ionise the sample molecules to produce (M+H) molecular ions [108, 113]. This type of ionisation is less energetic than EI and gives less structural information about a compound. The two ionisation techniques can be used to complement each other, particularly where the presence of a compound molecular ion is critical in establishing the identity of the compound. EI does not always yield molecular ions while CI does [113].

The mass spectrometer also has limitations. The salt and free base forms of certain compounds cannot be distinguished and similarly, it is not always possible to differentiate isomers of the same compound from each other [119, 120]. Certain GC columns have the capacity to differentiate between related compounds and so the combination of GC coupled to MS provide significant characterisation ability [121].

### **2.5.1 Sample inlet/Interface**

If the MS is already interfaced with a GC, then a transfer line/interface will be used to transport separated analyte molecules from the GC column into the MS for identification [113]. The interface is heated to allow the analytes to remain in their vaporised form. Heating also prevents the introduction of water molecules into the instrument [113]. In cases of stand-alone instruments, samples are introduced via an MS inlet, heated and vaporised before being swept into the ionising chamber [113].

### **2.5.2 Ionisation chamber/Ion source**

For GC-MS in EI mode the ionisation chamber comprises of an electron gun and an electron collector. Analyte molecules are ionised by being continuously showered with a beam of electrons from an electron gun (electron ionisation) [108, 111, 112].

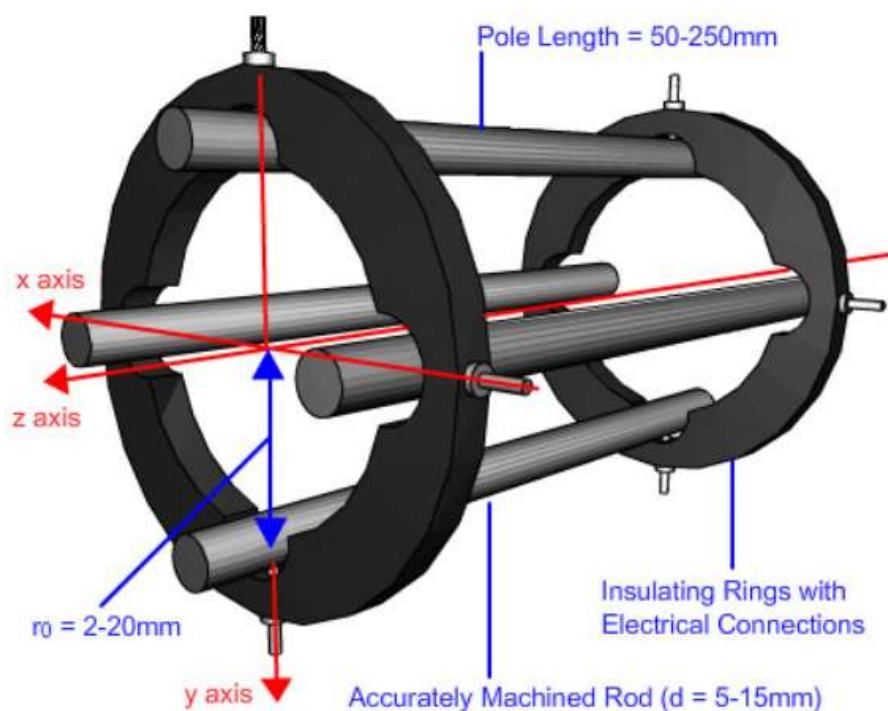
This results in the creation of a charged molecule and the associated loss of electrons. Electrons generated during ionisation are collected in an electron collector [113, 114].

### **2.5.3 Mass analyser**

The movement of charged analyte molecules through the analyser is controlled by placing different electrodes with different charges in the analyser [113, 119]. An electric field created by electrodes causes charged sample molecules to behave different from each other due to their mass where the rate of deflection is based on the mass and charge of the molecule [119]. This means that larger molecules reach the detector first because they are deflected less than those with lower mass. Some instruments have mass filters which dictate the order of molecules arriving at the detector and in these instruments smaller molecules may be detected first [113]. There are different mass analysers in the market; for this research a quadrupole mass analyser was used.

#### **2.5.3.1 Quadrupole mass analyser.**

Fragmented molecular ions and parent ions from the ionisation chamber pass through the quadrupole to reach the detector. The quadrupole is made of four metal rods arranged in a cylinder conformation (figure 2.3) [111, 119].



**Figure 2.3 Schematic diagram of a MS quadrupole [113]**

An alternating radio frequency (RF) is used to generate an electric potential across the rods during which acts to separate positively and negatively charged ions as they are carried into the mass analyser [8, 14]. The application of radio frequency also causes the ions to separate further according to their size. Smaller particles will be deflected to a greater extent as they move through the quadrupole and will reach the detector later than heavier particles [113, 119]. The application of direct current (DC) to the system favours the selection of smaller particles only and larger particles are deflected to a greater extent than smaller ones [119]. Smaller particles are refocused quicker and as a result reach the detector earlier [119]. By balancing the application of both the radio frequency and direct current to the mass analyser, the separation of the various fragments created from a given sample can be optimised.

#### **2.5.4 Detector**

Electrons received from the mass analyser are picked up by the detector and amplified to become electrical signals [114, 116]. Information about the sample can be acquired both in the Selective Ion Monitoring (SIM) mode which targets the analytes and/or scan modes [122].

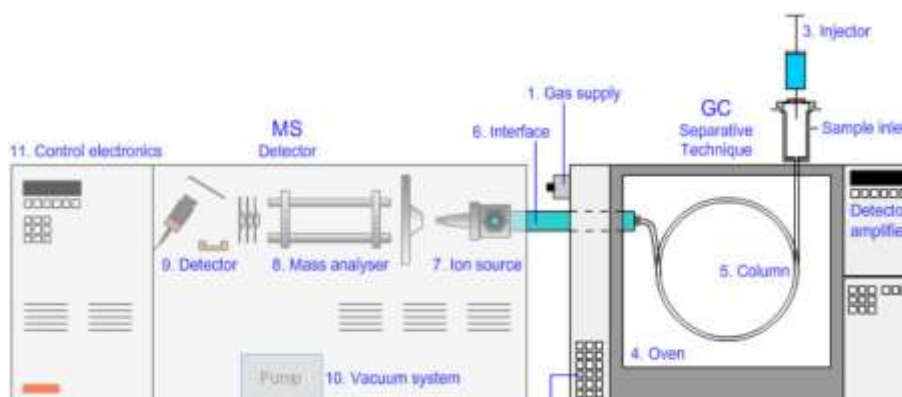
In SIM mode, the GCMS is set only to gather data on ions masses of interest. The advantages of this mode are that it enhances instrument sensitivity; helps eliminate sample matrix interferences facilitating lower analyte limits of detection and quantitation [122]. The scan mode is used for the identification of chemical components, quantitative analysis and also for the determination of some parameters for SIM analysis [122]. The scan mode was applied during the analysis of all samples for this research.

Signals produced by the detector are sent to a computer where a record of all data generated is produced [117, 118]. Information about each chromatogram can be searched and obtained from electronic libraries within the computer software. A plot of signals will reveal information about the sample such as analyte concentration (peak area) and composition [116-118].

#### **2.6 Gas Chromatography Mass Spectrometry (GCMS)**

GCMS is a combination technique comprising Gas Chromatography and Mass spectrometry through which complex substances are separated, identified and quantified [110, 113]. The basic layout of a typical GCMS instrument is presented in figure 2.4. A requirement for GCMS analysis is that samples should be both volatile and thermally stable [113]. Samples are usually analysed as organic solutions which are volatilised upon entry into the injection block of the instrument. As a result, materials of interest have to be solvent extracted and occasionally subjected to a series of wet chemistry techniques before analysis is possible [113]. Functionalised compounds may need to be derivatised before analysis; in order to minimise or

eliminate unwanted adsorption effects that may otherwise affect the quality of data obtained [113].



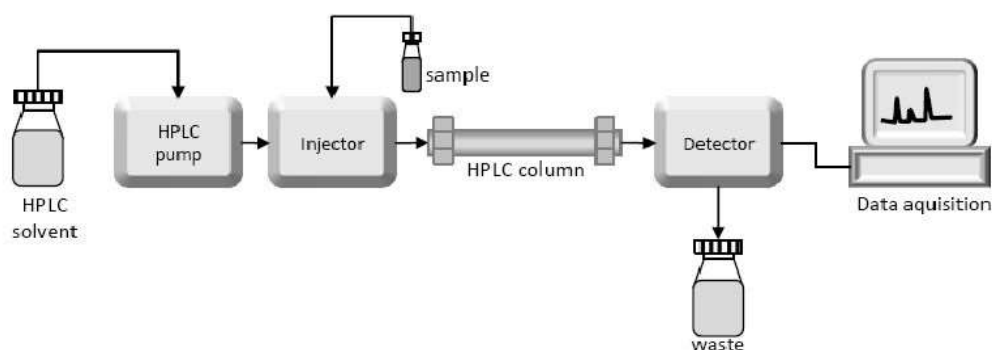
**Figure 2.4 Schematic diagram of a GCMS [113]**

## 2.7 High performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography is a separation technique which involves the injection of a small volume of a liquid sample into a column packed with particulate material (diameter size normally between 3 to 5  $\mu\text{m}$ ) [114, 115]; these make the stationary phase inside the column. Individual components of the sample are moved down the column as the liquid mobile phase is forced down the column by pressure from the instrument pump [114]. The liquid, which may also be used as the sample solvent, serves as the mobile phase. Separation of the components of the sample in the column involves various chemical and /or physical interactions between their molecules and the packing particles [123, 124]. The separated components are detected as they elute from the column based either on the fluorescence, refractive index or spectroscopic information [113, 114, 123, 124]. The time each component spends in the column is called its retention time; components with high affinity to the column will spend longer than those with less affinity [123]. A typical liquid chromatogram is shown in figure 2.5 below.



Five major components of a HPLC are the pump, injector, column, detector and computer.



**Figure 2.5 Schematic diagram of a HPLC [124]**

The HPLC pump is used to drive the mobile phase through the column at a specific rate (usually in mL/min). The normal flow rates for HPLC are in the 1 - 2mL/min range. Pumps are normally operated in pressure ranges of between 5800-7000 [110, 123]. HPLC pumps can either be isocratic or gradient based depending on the type of work under analysis [124]. The injector introduces the sample into the flow stream of the mobile phase; sample volumes are usually between 5 and 20 $\mu$ L [112]. Sample can be injected into the system manually via a syringe or automatically through the use of an auto sampler.

The data system which controls all the modules of the instrument and is also responsible for recording the retention times and amounts of individual components of the sample is usually run via dedicated software on a computer connected to the instrument [112, 123, 124].

Individual components of the sample that elute from the column are captured by the instrument detector. The detector response is sent to a recorder/computer where it is recorded as a chromatogram [124].

### **2.7.1 Gradient and Isocratic elutions**

Gradient elutions employ two or more solvent systems. After the elution has begun, the ratio of the solvents is varied in a programmed way, sometimes in a series of steps. This mode enhances separation efficiency. Isocratic elutions employ a single solvent or solvent mixture of constant composition. They are best suited for simple separations and often used in quality control applications while gradient conditions are normally used for complex samples such as unknown mixtures or during method development [112].

### **2.7.2 Separation modes of the HPLC**

Separation of the injected component mixture occurs within the analytical column using various physical and chemical parameters [113]. The nature of the stationary phase is responsible for causing the high backpressure at normal flow rates [124]. The pump must push hard to move the mobile phase across the column and therefore creates resistance increasing the pressure inside the system [123].

The HPLC uses four separation modes for most components which are reversed phase, normal phase, ion exchange and size exclusion chromatography [112, 113, 124].

Reversed phase chromatography is the most commonly encountered stationary phase mode mostly due to its versatility; it can be used for polar, non-polar, ionisable and ionic molecules. Column packaging for this mode is non-polar and the mobile phase is normally a buffer and a water miscible organic solvent like acetonitrile or acetone [112, 123, 124]. Samples containing a wide range of components can easily be separated via gradient elution with this mode.

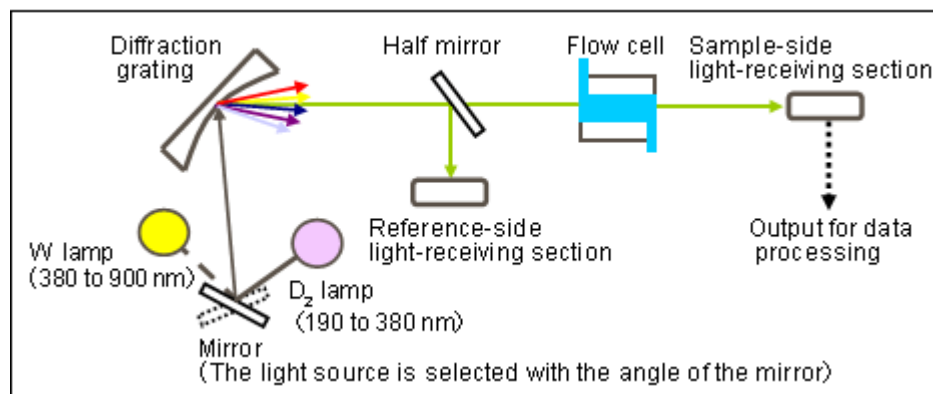
The stationary phase for normal phase chromatography is polar (e.g. silica gel) while the mobile phase is non-polar. These are less common and used for the analysis of water sensitive compounds, chiral compounds, separation of various lipid classes, as well as cis-trans isomers [119].

The column packing for ion exchange chromatography contains ionic groups and the mobile phase is an aqueous buffer [110]. This mode is mostly suitable for the separation of organic and inorganic ionic compounds and cations in aqueous solutions [110]. It is also suitable for the separation of ionic dyes. Separation of proteins can also be achieved with ion exchange chromatography. The last separation mode for HPLC is size exclusion chromatography. In this mode, the sample and the column packing materials do not interact; instead molecules diffuse into the pores of a porous medium [110, 123]. Separation is based on the relative size of the sample molecules to the pores of the medium. This mode is mainly used for polymer characterisation and for protein analysis [123].

### **2.7.3 HPLC detection systems – Ultra Violet (UV) and Diode Array Detector (DAD)**

There are many detection modes used for HPLC but the three most common are spectroscopic, refractive index and fluorescence detections [18-20]. The detection system used for this research was the Diode Array Detector (DAD).

Spectroscopic (UV or UV/UV-VIS) detectors are most frequently used to measure components showing an absorption spectrum in the ultraviolet or visible region [112, 124]. These employ a deuterium discharge lamp ( $D_2$  lamp) as a light source, with the wavelength of its light ranging from 190 to 380 nm. Measurement of sample components at wavelengths longer than this uses a UV-VIS detector which employs an additional tungsten lamp with wavelength range of 320- 1100nm [124]. When using these detectors, light from the lamp is shone onto a diffraction grating and dispersed according to the wavelength at which measurement is required [124]. For instance, when the measurement is performed with a wavelength of 320 nm, the angle of the diffraction grating is adjusted so that 320 nm light is shone on the flow cell [124]. Comparison of the differences in light intensity between light emerging from the flow cell after interacting with the sample and the reference light which has not interacted with the sample can be determined and output as absorbance [124] (figure 2.6). In some cases a UV spectrum will also be generated and can be used to identify the compound (s) [112, 123, 124].



**Figure 2.6 Illustration of a UV-VIS detector optical system [124]**

While a UV-VIS detector has only one sample-side light-receiving section, a Diode Array Detector (DAD) has multiple (1024 for L-2455/2455U) photodiode arrays to obtain information over a wide range of wavelengths concurrently [124]. Spectra are measured at intervals of 1 second or less during separation by HPLC with continuous eluate delivery. If the measurement is performed at a fixed wavelength, components are identified from only their retention time; thus, a minor deviation in retention time can make identification of components difficult [124]. In such a case, the DAD can be used to identify components by a comparison of the spectrum [124].

The difference between DADs and UV-VIS detectors is that light from the lamps is shone directly onto the flow cell, light that passes through the flow cell is dispersed by the diffraction grating, and the amount of the dispersed light is estimated for each wavelength [124] (figure 2.7). Noise can be a feature in DAD output because the amount of light is small; the DAD is also susceptible to various changes, such as lamp fluctuations, because the reference light cannot be received [124-128].

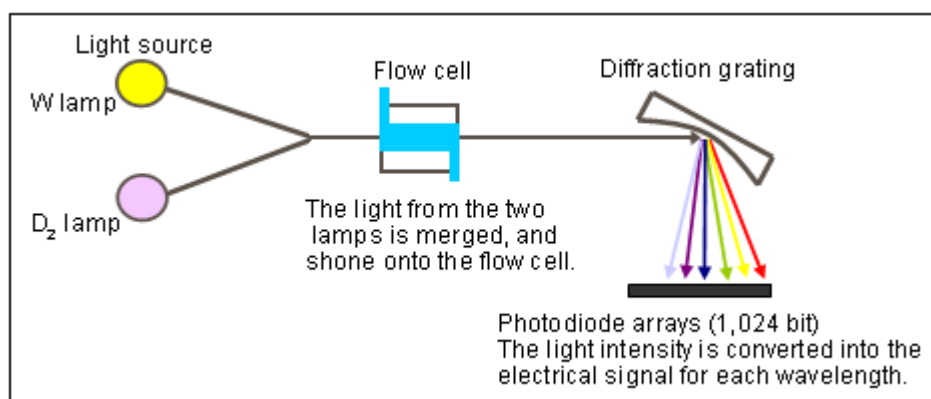


Figure 2.7 Schematic diagram of Diode Array detector [124]

## 2.8 HPLC detection systems – relating signal to concentration

When light passes through the compound, energy from the light is used to promote an electron from a bonding or non-bonding orbital into one of the empty anti-bonding orbitals [126] (figure 2.8).

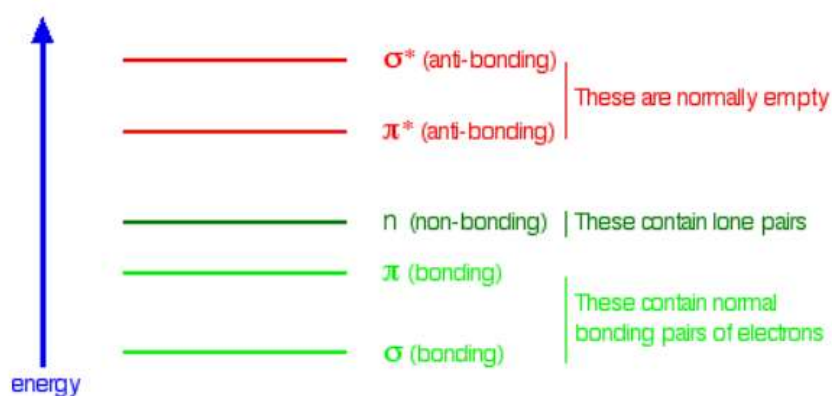


Figure 2.8 Energy level diagram [127]

Possible electron jumps that light might cause is shown in figure 2.9.

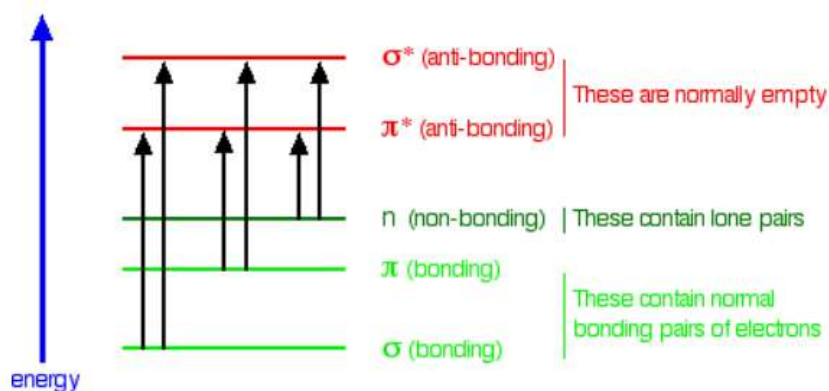


Figure 2.9 Different electron excitation possibilities [127]

In each possible case, an electron is excited from a full orbital into an empty anti-bonding orbital. Each jump takes energy from the light, and a larger jump obviously needs more energy than a small one. Each wavelength of light has a particular energy associated with it, if that particular amount of energy is just right for making one of these energy jumps, then that wavelength will be absorbed - its energy will have been used in promoting an electron [127].

An absorption spectrometer works in a range from about 200 nm (in the near ultra-violet) to about 800 nm (in the very near infra-red). Only a limited number of the possible electron jumps absorb light in that region. In order to absorb light in the region from 200 - 800 nm (where the spectra are measured), the molecule must contain either pi bonds or atoms with non-bonding orbitals [127].

## 2.9 Chromophores

Many compounds absorb ultraviolet (UV) or visible (Vis.) light. These are called chromophores; the characteristic feature of chromophores to absorb light of UV and visible wavelengths from 200 nm to 400 nm and from 400 nm to 800 nm respectively can be used to determine their concentration by absorption photometry. The method measures the decrease in light intensity when light passes through a coloured solution. The distance which the light has to pass through a solution is called the path length. With a linearly rising concentration of the chromophore solution, the intensity of the emergent beam of light falls off

exponentially [127]. The amount of radiation absorbed may be measured in a number of ways:

- i. Transmittance,  $T = P / P_0$   
% Transmittance,  $\%T = 100 T$

- ii. Absorbance,  
 $A = \log_{10} P_0 / P$   
 $A = \log_{10} 1 / T$   
 $A = \log_{10} 100 / \%T$   
 $A = 2 - \log_{10} \%T$

Where:

$I$  = intensity of transmitted light

$I_0$  = intensity of incident light

$T = I/I_0$  = transmittance

### **Equation 2.2 Equation for absorbance [128]**

Using the last equation,  $A = 2 - \log_{10} \%T$ , absorbance can be easily calculated from percentage transmittance data, but this is not normally used because the relationship between the two variables is not linear. The Beer Lambert equation [2.3] is mostly preferred. With the equation, the relationship between absorbance is linear to the concentration and the path length. If the molar absorption coefficient of a chromophore in solution is known then its concentration can be calculated using the Lambert-Beer's law equation:

$$A = \epsilon \times c \times d$$

Where

$A$  = absorbance (dimensionless)

$c$  = concentration (mol/L)

$d$  = path length (cm)

$\epsilon$  = molar absorptivity coefficient (L/mol x cm)

### **Equation 2.3 Lambert-Beer's law [128]**

A plot of absorbance against concentration using the Lambert-Beer's law yields a straight line passing through the origin. However the Beer-lambert's law is limited since it is only valid for highly diluted solutions [128].

## **2.10 Quantification methods using GCMS and HPLC**

Quantitation of samples using both the GC-MS and HPLC instruments uses chromatographic data to determine the amount of a given component in a mixture. The data can be in the form of either peak height or peak area obtained from an integrated chromatogram [129]. Data used should be gathered accurately; peak resolution should be ensured to accurately determine the size or height. Several types of quantitation methods exist; but commonly used ones are area percent, single point external standard, multiple point external standard, single point internal standard, and multiple point internal standard [129]. Multi point external standard quantitation method was used for this research.

### **2.10.1 Multi point external standard Method**

Multi-level calibration can be used when it is not sufficiently accurate to assume that a component shows a linear response or to confirm linearity of the calibration range, otherwise a single point external standard method is applied [130]. A sample containing a known amount of analyte or analytes is first analysed, followed by calculation of its response. Equation 2.4 is used to calculate the response factor [129, 130].

$$\text{Response factor} = \text{Peak area/standard amount}$$

**Equation 2.4 Calculation of analyte response factor [129]**

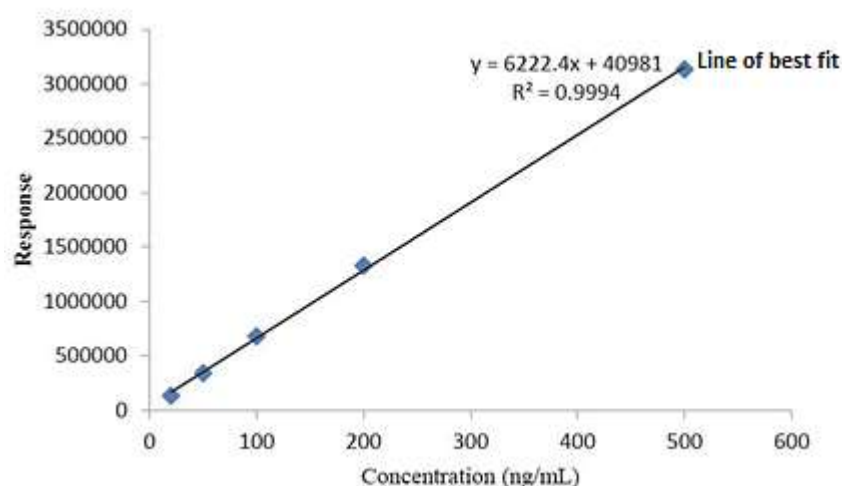
This is followed by the analysis of a sample with an unknown concentration and recording of the peak area. The amount of the analyte in the sample is then calculated using equation 2.5 [129].

$$\text{Analyte amount} = \text{Peak area/response factor}$$

**Equation 2.5 Calculation of analyte concentration [129]**



After calculating concentrations for each analyte at different calibration levels, a line fitting algorithm such as point to point, linear least squares, or quadratic least square is used to produce a calibration curve [129] (figure 2.10).



**Figure 2.10 Typical calibration curve [131]**

Linear regression attempts to model the relationship between two variables by fitting a linear equation to observed data. One variable is considered to be an explanatory variable, and the other is considered to be a dependent variable [132]. Linear regression calculates the equation that minimises the distance between the fitted line and all data points [132]. From the calibration curve, a regression analysis will yield an equation which describes the best fit of the line through the data points, with the form:

$$Y = mx + c$$

Where:

$Y$  = peak area

$m$  = the slope of the regression line

$c$  = intercept of the regression line with the y-axis [132]

The slope of the calibration line is often used to determine the sensitivity of the analytical method while the intercept indicates the degree of systematic error within the method and is the direct result of 'background' response [132].

### **2.10.2 Least-Squares Regression**

The most common method for fitting a regression line is the method of least-squares. This method calculates the best-fitting line for the observed data by minimizing the sum of the squares of the vertical deviations from each data point to the line. Because the deviations are first squared, then summed, there are no cancellations between positive and negative values [133].

## **Chapter 3: METHOD DEVELOPMENT AND VALIDATION STUDIES**

### **3.0 Gas Chromatography Mass Spectroscopy methods for efavirenz (EFV)**

There are no available gas chromatographic analytical methods reported in the literature for efavirenz (EFV). As a consequence a new GC method was developed and validated. This process was divided into two phases; the first phase included the determination of initial instrument conditions, sample preparation, system suitability and selectivity. The second phase was involved with the validation of the developed method. Since cannabis is one of the alleged ingredients of nyaope, a GC-MS protocol developed by Cadola [134] for the analysis of cannabis was used as an initial starting point.

#### **3.1 Chemicals and reagents**

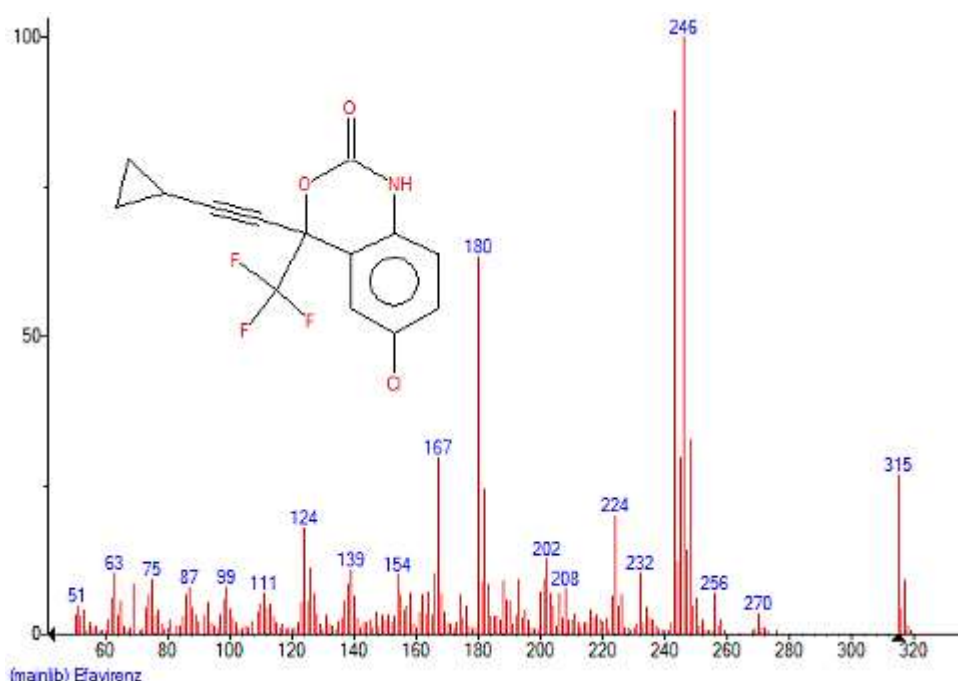
Efavirenz reference standard was purchased from Sigma Aldrich (Gillingham, Dorset, UK). Caffeine, Nevirapine and 2-(4-Isobutylphenyl) propanoic acid (Ibuprofen) reference standards were all also purchased from Sigma Aldrich (Gillingham, Dorset, UK). The Grob test mix was purchased from Restek, UK. Efavirenz tablets (600mg) were donated by Pharma South Pharmacy (Gaborone, Botswana).

#### **3.2 Determination of initial operational conditions for GCMS**

An Agilent 7890A gas chromatograph interfaced with an Agilent 5977E mass selective detector was used. Separation was achieved on a standard non-polar DB-1 column, (15 m length, 0.32 mm i.d and 0.25  $\mu\text{m}$  film thickness). Sample injections were made in a split mode using a general purpose split/split-less liner packed with glass wool. The GC oven temperature program started at 100<sup>0</sup>C, held for 1 minute and increased to 260<sup>0</sup>c at a rate of 10<sup>0</sup>C min<sup>-1</sup> and held for 10 minutes resulting in a total run time of 27 minutes. Sample volumes of 1 $\mu\text{L}$  were injected into the instrument at a split ratio of 10:1 using helium as a carrier gas. The flow rate of the helium was set at a constant flow of 20 $\mu\text{L}$  min<sup>-1</sup>. Other temperature settings were as follows; the injector was set at 280<sup>0</sup>C, transfer line at 250<sup>0</sup>C, ion source at 230<sup>0</sup>C

and the quadrupole at 150<sup>0</sup>C. Data acquisition was performed in full scan mode. Compounds were identified using both the NIST 2014 mass spectral database and data from available literature [134- 138]. Tuning of the MS was performed once a month using PFTA.

For identification purposes, a standard solution of 1mg/mL EFV in methanol was prepared. Three replicate injections of the solution were performed using the above settings. Efavirenz was detected at 16.96 minutes and the mass spectrum (figure 3.1) reveals the main ions identified at  $m/z$  315, 246, 243, 224, 180 and 167.



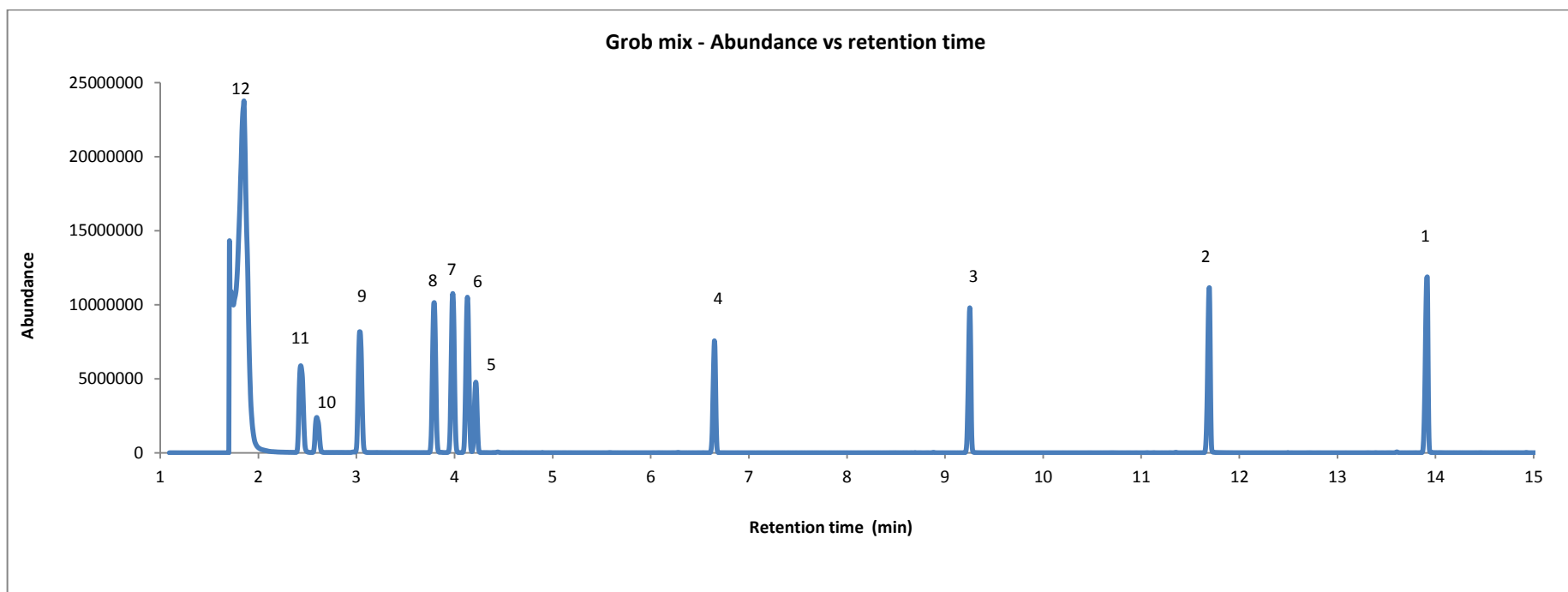
**Figure 3.1 efavirenz mass spectrum**

Column efficiency test and instrument precision were then performed to establish system suitability for analysis of the target analyte. A comprehensive, standardised quality test for column efficiency was proposed by Grob and Grob in 1978 [142] testing. For this study, a commercial test mix based on that proposed by the Grobs [142] was used to test the GC column efficiency. The test mix comprised a total of 13 hydrocarbon components at a concentration of 0.05% V/V each in methyl

chloride. Six GC replicate injections of 1 $\mu$ L test mix were performed. A chromatogram obtained from the test mix is presented in figure 3.2.

**Key:**

(1)Eicosane (2)octadecane (3)hexadecane (4)tetradecane (5)Dodecane (6)1,3 ethyl methylbenzene  
(7)1,3,5 tri methyl benzene (8)ethyl methyl benzene (9)Decane (10)p-Xylene (11)octane (12)toluene



**Figure 3.2 Grob mixture chromatogram**

The peak width at half height was calculated for each component across the six injections. The number of theoretical plates for each component was then calculated using equation 1 and results used as a true representation of the total number of plates that each component. The results (table 3.1) show high theoretical number plate values for most components with the exception for p-xylene and octane.

**Table 3.1 Theoretical plate number for components of the Grob test mix**

<b>Assigned number on chromatogram</b>	<b>Component</b>	<b>Theoretical Plate number</b>
1	Eicosane	5231
2	Octadecane	3657
3	Hexadecane	8361
4	Tetradecane	4869
5	Dodecane	6475
6	1,3 ethyl methyl benzene	2458
7	1,3,5 tri methyl benzene	5034
8	1 ethyl 3 methyl benzene	9769
9	Decane	20585
10	P-xylene	896
11	Octane	646
12	Toluene	1757

### **3.2.1 Instrument precision**

The International Conference on Harmonization (ICH) defines precision as “the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under a set of prescribed conditions” [144]. Precision can also be defined as a measure of random errors in a method [145].

There are three levels at which precision may be expressed and these are;

- (i) Repeatability - defined as a measure of the closeness of the agreement between reciprocally independent test results obtained using the same method, on identical test material in the same laboratory, performed by the same operator using the same equipment on the same day [145].

- (ii) Variations in the conditions for repeatability when performed within the same laboratory results with intermediate precision.
- (iii) Reproducibility is used to assess the closeness of agreement between results obtained with the same method on identical test material in different laboratories with different operators using different equipment [144-146].

Precision is concentration dependent and therefore should be measured at different concentrations within the working range, typically at the lower, mid and upper parts [146]

For this research, the repeatability factor was used to measure precision. Within day instrument repeatability was assessed by performing 5 replicate injections of both the Grob test mixture and a 1mg/mL of the efavirenz (EFV) standard in methanol. Mean, standard deviation and % relative standard deviation (%RSD) values of peak areas across the six injections were calculated and evaluated. Results obtained for both solutions were within  $\leq 5\%$  [147, 148] confirming a very good repeatability and that the instrument was suitable for the intended analytical work. The results are displayed in Table 3.2 and 3.3



**Table 3.1 Analytical repeatability results for the test mix**

Test mix analyte	Peak area					Mean	SD	RSD %
	Injection 1	Injection 2	Injection 3	Injection 4	Injection 5			
<b>Toluene</b>	135866973	140852156	135866973	138147943	144716169	<b>139090043</b>	<b>3755737.2</b>	<b>2.7</b>
<b>Octane</b>	46744731	48296183	46744731	47764416	49146908	<b>47739394</b>	<b>1033252.7</b>	<b>2.16</b>
<b>p-Xylene</b>	186167822	189822620	186167822	188035602	193503239	<b>188739421</b>	<b>3065488.8</b>	<b>1.62</b>
<b>1,3 ethyl methyl benzene</b>	222075119	223059097	222075119	221540433	229634987	<b>223676951</b>	<b>3375513.9</b>	<b>1.53</b>
<b>1 Ethyl -2 methyl benzene</b>	236155881	236015369	236155881	235279186	243069521	<b>237335168</b>	<b>3226188.6</b>	<b>1.36</b>
<b>1,3,5 ethyl methyl benzene</b>	226859436	225603633	226859436	224831466	233078876	<b>227446569</b>	<b>3265253</b>	<b>1.44</b>
<b>Decane</b>	93691629	93986694	93691629	93413314	96811353	<b>94318924</b>	<b>1407986.7</b>	<b>1.49</b>
<b>Dodecane</b>	140225119	140225119	140225119	140228653	146292923	<b>116205387</b>	<b>57162176</b>	<b>1.89</b>
<b>Tetradecane</b>	176181129	176819773	176181129	176594897	183121330	<b>177779652</b>	<b>2998711.7</b>	<b>1.69</b>
<b>Hexadecane</b>	202413224	202048768	202413224	203686965	209227344	<b>580567133</b>	<b>845069770</b>	<b>1.48</b>
<b>Octadecane</b>	216779465	214487216	216779465	215977681	221716745	<b>217148114</b>	<b>2720040.7</b>	<b>1.25</b>
<b>Eicosane</b>	234546438	232060443	234546438	233434373	239989219	<b>234915382</b>	<b>3014967.9</b>	<b>1.28</b>

Table 3.3 EFV analytical repeatability test results

<b>Replicate</b>	<b>efavirenz peak area</b>
1	118117451
2	125124730
3	120992717
4	116694158
5	123852796
6	111606910
<b>Sum</b>	<b>716535468</b>
<b>Average</b>	<b>119422578</b>
<b>SD</b>	<b>11203696</b>
<b>RSD%</b>	<b>4.18%</b>

### 3.3. GCMS Method Validation

A method validation is performed to ensure that data generated from instrumental analysis can be relied upon forming an important part of any good laboratory practice [144]. The process of validating a method can also be described as “establishing documented evidence that provides a high degree of assurance that a specific method, and ancillary instruments included in the method, will consistently yield results that accurately reflect the quality characteristics of the product tested” [145]. Many reasons can be advanced towards the validation of analytical methods; the most important one being to meet the requirements and validation characteristics included in both the ISO/IEC 17025 standard and the International Conference on Harmonization (ICH) guidelines on method validation.

The ISO/IEC 17025 is the most relevant standard for chemical analytical laboratories including forensic science laboratories [149]. ICH is interested and involved with the harmonisation of technical requirements for the registration of products among the European Community, Japan and the United States of America [145, 146]. Typical validation parameters considered for qualitative method validation include linearity, specificity/selectivity, precision, limit of detection, limit of quantification, accuracy, and range and analyte stability

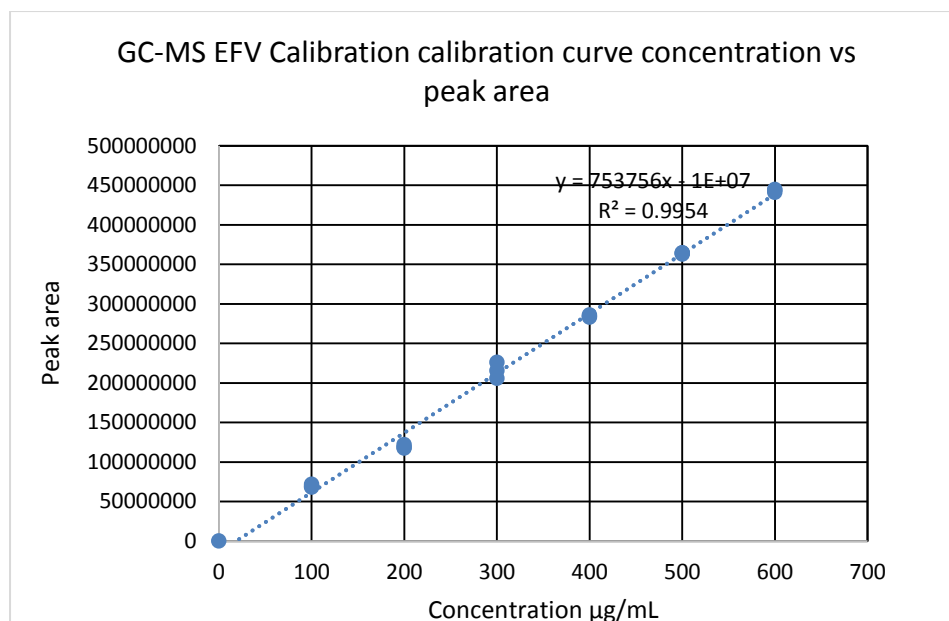
### 3.3.1 Method Linearity

The linearity of a method is defined as its ability to obtain test results that are directly proportional to the amount of analyte in the sample within a specified range. It is used to study the relationship between the target analyte concentration and the mass detector response [145]. A linear response will therefore allow for its quantification of the analyte through either the peak area or height.

To assess linearity, three standard stock solutions of 1mg/mL of efavirenz (EFV) in methanol were prepared. Aliquots of these solutions were further diluted with methanol to make six different solutions with concentrations ranging from 100-600 µg/mL. Three replicate injections of these solutions were performed and their response (table 3.4). Data obtained from these were subjected to regression analysis using the least squares method to construct a calibration curve (figure 3.3).

**Table 3.4 Peak areas and %RSD values for three EFV replicate injections across the chosen calibration range**

EFV standard replicate	100µg/mL	200µg/mL	300µg/mL	400µg/mL	500µg/mL	600µg/mL
1	71607690	117284724	225903704	284391686	364589472	440701756
2	67930993	118878740	205618390	285863720	364376878	444748825
3	69177128	121851640	215573165	282997920	362940605	443778088
<b>Average</b>	<b>69571937</b>	<b>119338368</b>	<b>215698419.7</b>	<b>284417775</b>	<b>363968985</b>	<b>443076223</b>
<b>SD</b>	<b>1869875</b>	<b>2317892.116</b>	<b>10143237.04</b>	<b>1433078.1</b>	<b>896924.26</b>	<b>2112854.2</b>
<b>RSD%</b>	<b>2.69</b>	<b>1.94</b>	<b>4.70</b>	<b>0.50</b>	<b>0.25</b>	<b>0.48</b>



**Figure 3.3 GCMS efavirenz calibration curve**

The correlation coefficient ( $R^2$ ) of efavirenz in methanol was found to be 0.995, confirming a linear relationship between the analyte and concentration. %RSD values for linearity concentrations ranged between 0.2-4.7percent (Table 3.4).

### 3.3.2 Analytical Range

The range of an analytical method is the interval from the upper to the lower levels (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. Analytical range is normally expressed in the same units as the test results obtained by the analytical method [140, 144, 147]. Based on the instrument calibration results, the developed method demonstrates linearity between EFV concentrations of 100-600 $\mu\text{g/mL}$ .

### 3.3.3 Method Selectivity

A selective method is said to be the one in which the presence of other components in the sample mixture does not affect the identification and/or quantification of the analyte of interest. The other components may include impurities, degradation products, sample matrix and/or other components with similar behaviour [150-53].

To assess the selectivity, stock solutions of efavirenz, 2-[4-(2-methylpropyl)phenyl]propanoic acid (ibuprofen), caffeine and nevirapine were prepared by weighing 5mg of each standard and dissolving in 5mL of methanol to yield 4 standard solutions, each with a concentration of 1mg/mL. The two standards of caffeine and ibuprofen were chosen based on the consideration that they likely to be present in most human biological samples as they are thought to be used by most people. Nevirapine on the other hand is an anti-retro viral drug which, in most cases, has been observed to be taken in combination with or in place of EFV.

Following the preparation of the three stock solutions, a laboratory standard mixture was prepared by mixing together 1mL each of the prepared stock solutions. 6 replicate injections of the prepared standard were analysed. The standard mixture was very well separated (figure 3.4) with RSD values <5% for each compound in the mixture (Table 3.5). None of the components eluted at the retention time of efavirenz, confirming that the method was selective for this target analyte.

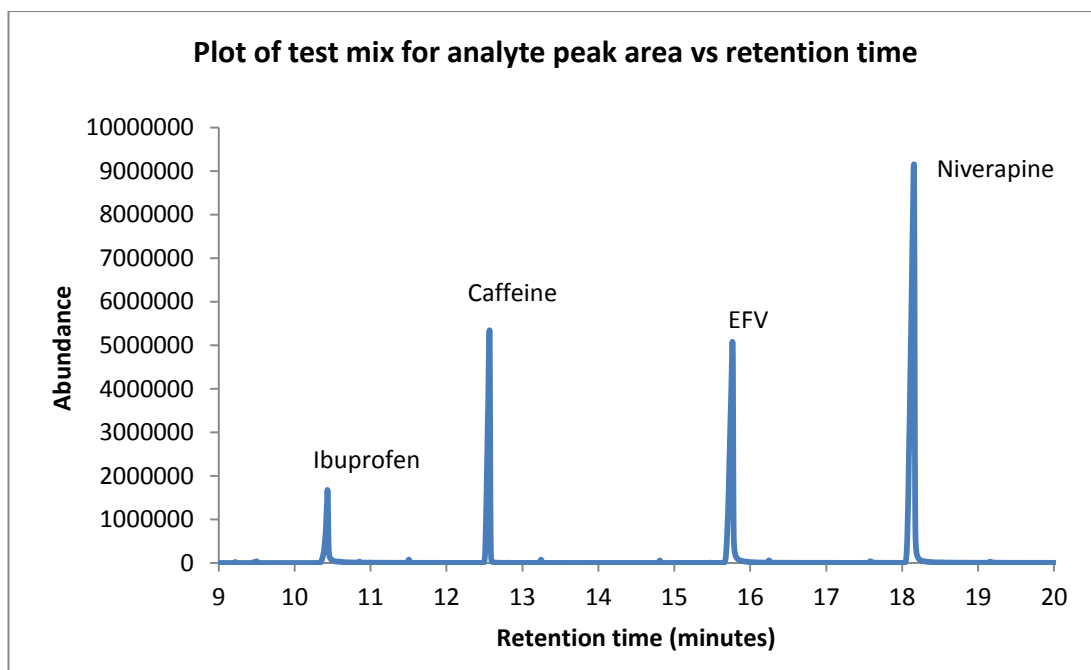


Figure 3.4 Chromatogram of the laboratory standard mixture

Table 3.5 %RSD values of peak area for EFV mixed with 3 standards (ibuprofen, caffeine and nevirapine) using GCMS

Injection	Ibuprofen	Caffeine	efavirenz	Nevirapine
1	25328192	108613924	118264157	258845222
2	25373055	11973163	125124730	272723489
3	26121465	112011811	120992717	265649888
4	25724251	110381326	116694158	258998759
5	24426022	123094098	123852796	279989405
6	23010254	105742316	111606910	249006997
<b>Mean</b>	<b>24997206.5</b>	<b>111969440</b>	<b>119422578</b>	<b>264202293</b>
<b>SD</b>	<b>1124063.73</b>	<b>5938324.541</b>	<b>4989540.793</b>	<b>11050893.3</b>
<b>%RSD</b>	<b>4.50%</b>	<b>5.30%</b>	<b>4.18%</b>	<b>4.81%</b>

### 3.3.4 Analyte stability

Potential analytical bias during method development can be eliminated by investigating the stability of the target analytes and standards used [152, 153]. Bias can arise at the start of an analytical investigation due to degradation/decomposition of chemical compounds during sample preparation

procedures and storage of prepared aliquots or while awaiting analysis in instrument auto samplers [150].

Analyte stability can be defined as the measure of the capacity of an analyte to remain within established specifications (identity, concentration and quality) throughout the whole analytical procedure [144-146] and is mainly dictated by analyte storage conditions (before, during and after analysis). Stability tests are carried out by comparing freshly prepared analyte solutions of known concentration with similar samples retained for different periods of time under various conditions [146].

Stability was assessed by analysing two sets of EFV standard solutions at concentrations 200, 300 and 500µg/mL over 9 consecutive days. One set of standard solutions were maintained at room temperature (19°C) while the other was kept in the fridge (2.5°C) for the duration of the test period (9 days). Three replicate injections were performed daily of each sample. Daily mean peak areas at each concentration were calculated from which graphs of peak area vs number of days were generated. Percentage relative standard deviations for each concentration across the three replicate injections were calculated. The results obtained are presented in tables 3.6, 3.7 and figures 3.5.

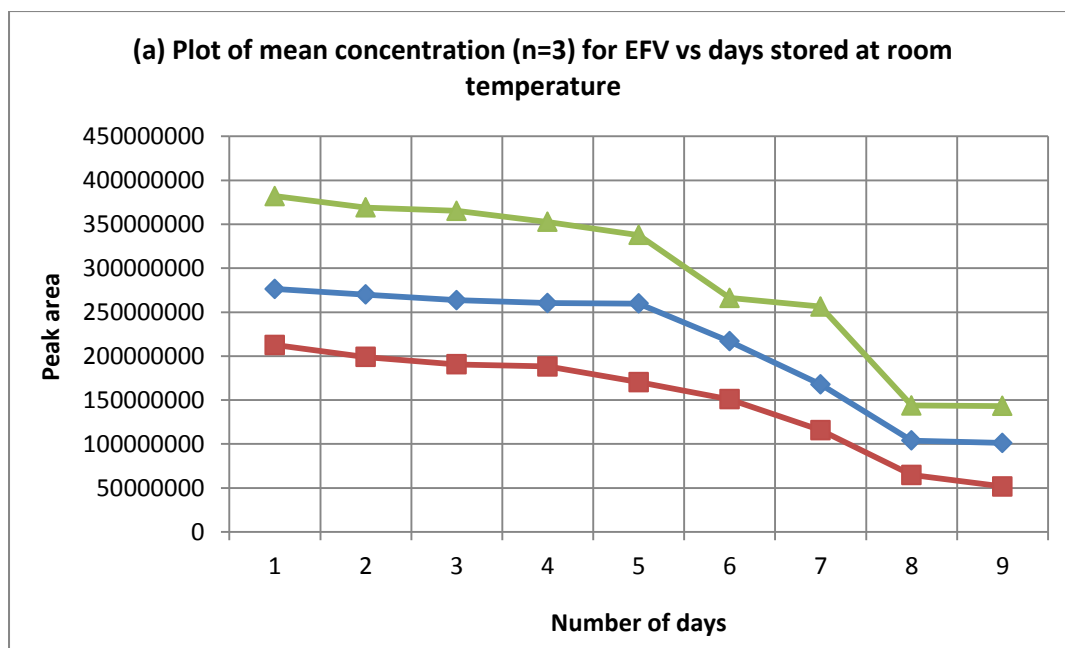
**Table 3.6 %RSD results for analyte stability test at room temperature performed over 5 days**

Standard solution concentration		Average peak area (n=3)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Mean	SD	%RSD
500µg/mL	371884643	368948416	365096057	352547121	337714249	359238097	14111930	3.93
300µg/mL	276296770	270024795	263645980	260245309	259729836	265988538	7074507	2.66
200µg/mL	202513340	198893706	190580607	188145291	180410062	192108601	8791966	4.58

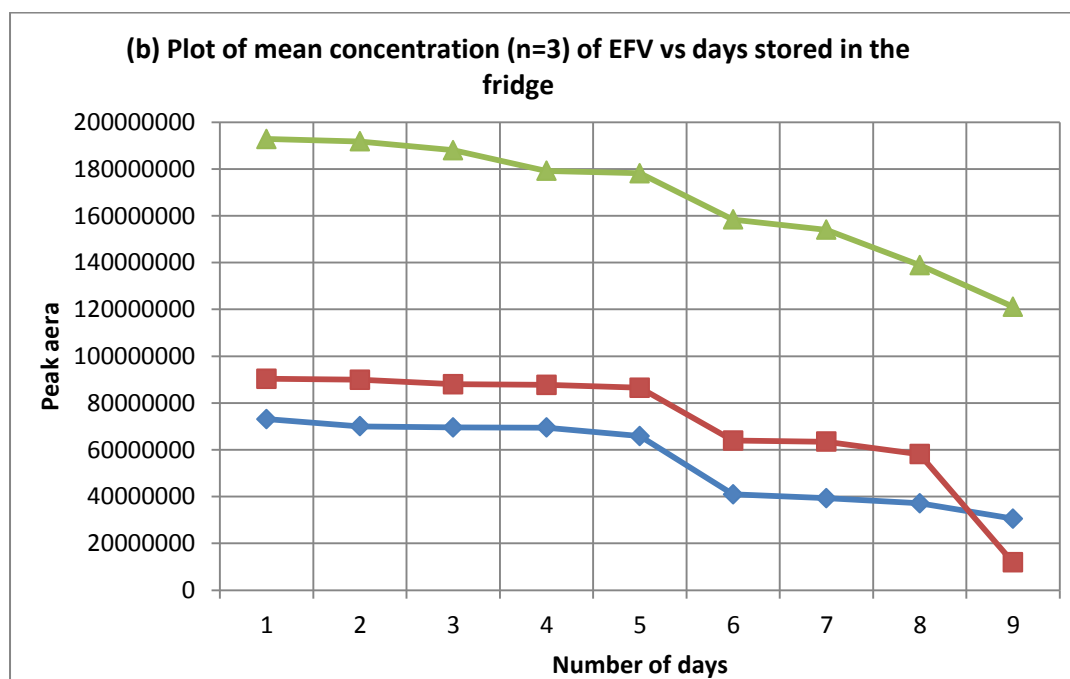
**Table 3.7 %RSD results for average analyte stability for samples stored in the fridge over 5 days**

Standard solution concentration		Average peak area (n=3)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Mean	SD	%RSD
500µg/mL	192840944	191814446	188040105.7	179230230.7	178152602	186015666	6199654	3.33
300µg/mL	90350795.33	899949994.67	88014750.3	87775070	86494363	88516995	1435970	1.62
200µg/mL	73089827	17000950	69555841	69459549	65881877	69597609	2288482	3.29





\*Key: green = 500µg/mL, blue = 300µg/mL, red = 200µg/mL



**Figure 3.5 Plot of EFV stability at a) room temperature and b) samples kept in the fridge for different concentrations (200, 300 and 500µg/mL) of methanol**

\*Key: green = 500µg/mL, red = 300µg/mL, blue = 200µg/mL

The data reveal that both room temperature and fridge samples remained relatively stable for five days. Stability was determined by the %RSD for the average peak area where all three solutions remained within the  $\leq 5\%$  range.

### 3.3.5 Limit of Detection (LOD) and Limit of quantitation (LOQ)

The limit of quantification is the lowest concentration of analyte detectible and quantifiable with certainty of precision and accuracy using the analytical method. LOD is the lowest amount of analyte that can be detected without being necessarily quantified as an exact value. It can also be described as the lowest concentration that can be distinguished from the background noise with a certain degree of confidence [144, 146, 150]. LOQs are normally used as a measure of the amount of impurities and/or degradation products in a sample [147, 148].

The LOD and LOQ were derived from the standard deviation of the response and slope of the calibration curve using Equations 3.2 and 3.3 below;

$$\text{LOD} = 3.3 \times (\text{Standard Deviation of the analyte response} / \text{slope of the Calibration curve for the analyte}).$$

**Equation 3.2 LOD calculations [147]**

$$\text{LOQ} = 10 \times (\text{Standard Deviation of the analyte response} / \text{slope of the Calibration curve for the analyte}).$$

**Equation 3.3 LOQ calculations [147]**

Based on equation 3.2 the LOD for EFV was calculated as:

$$3.3 \times 5494506 / 743247 = 24 \mu\text{g/mL}.$$

The LOQ for EFV (base on equation 3.3) was found to be:

$$10 \times 5494506.47 / 743247 = 74 \mu\text{g/mL}$$

### 3.3.6 Method accuracy

Accuracy is used to estimate how close the method test results are to the true response value of the analyte in the sample [145]. Disagreements between the two results may arise from either systematic or random errors or both [150].

Accuracy can be obtained through several ways;

- (i) By comparing the results from the method with those obtained from an established reference method, assuming that the uncertainty of the reference method is known [147].
- (ii) By injecting known concentrations of sample (either a control sample or certified reference material) and comparing the measured value with the true value. If neither of these is available then a spiked blank sample matrix can be used [144, 145, 147].
- (iii) By comparing the response of the extract with the response of the reference material dissolved in a pure solvent (percentage recovery). This can also be used to assess the effectiveness of sample preparation [144, 145, 147]

The accuracy of the new method was estimated by injecting known concentrations of sample certified reference material and comparing the measured value with the true value. The accuracy of the new method was found to be  $99.4 \pm 6.3\%$ . Percentage analyte recovery using the new method developed for this study was found to be very good (over 85%) (Table 3.8 and 3.9)

**Table 3.8 Method analyte recovery at each calibration level -EFV standard sample**

<b>Actual sample concentration (µg/mL)</b>	<b>Peak Area</b>	<b>calculated concentration (µg/mL)</b>	<b>Calculated accuracy (based on peak area) (%)</b>
100	71607690	110	110
100	67930993	105	105
100	69177128	107	107
200	117284724	171	87
200	118878740	173	87
200	121851640	177	89
300	225903704	317	106
300	205618390	290	97
300	215573165	304	101
400	282997920	396	99
400	284391686	398	100
400	285863720	394	99
500	364589472	504	101
500	364376878	504	101
500	362940605	502	100
600	440701756	606	101
600	444748825	612	102
600	443778088	611	102
<b>Mean recovery</b>			<b>99.4%</b>
<b>SD</b>			<b>6.3</b>

**Table 3.9 calculated %RSD for recovery studies**

<b>Replicate</b>	<b>100µg/mL</b>	<b>200µg/mL</b>	<b>300µg/mL</b>	<b>400µg/mL</b>	<b>500µg/mL</b>	<b>600µg/mL</b>
1	100	86	106	99	101	101
2	105	87	97	100	101	102
3	107	89	102	100	100	102
<b>Mean</b>	<b>107</b>	<b>87</b>	<b>101</b>	<b>99</b>	<b>101</b>	<b>102</b>
<b>SD</b>	<b>2.52</b>	<b>1.56</b>	<b>4.55</b>	<b>0.48</b>	<b>0.24</b>	<b>0.47</b>
<b>%RSD</b>	<b>2.34</b>	<b>1.79</b>	<b>4.49</b>	<b>0.49</b>	<b>0.24</b>	<b>0.47</b>

Summary of GCMS method validation results is presented in table 3.10 below

Table 3.9 Summary of GCMS method validation results

Parameter	Results
Linearity	100-600µg/mL
Cor. Co-efficient	0.99
Precision (%RSD): intraday repeatability	1-4%
Method specificity (%RSD) efavirenz with three other drugs	4-6%
Method accuracy	99-105%
LOD	24µg/mL
LOQ	74µg/mL
Analyte stability (%RSD) fridge (5 days) Room temperature (5 days)	1-4 % 4-5%

These combinations of results provide confidence that the chosen Gas Chromatography Mass Spectrometry method for the detection of EFV was appropriately validated.

#### 3.4.0 HPLC Method adoption and validation

An analytical method developed by Veldkamp *et al* [154] for the analysis of EFV in biological fluids was adopted. A method revalidation was performed due to the differences in the sample matrices (biological fluids vs EFV powdered tablet). As with the GCMS validation, the process was divided into two phases which included the establishment of instrumental conditions, sample preparation, system and selectivity optimization followed by validation of the developed method.

##### 3.4.1 Chemicals and reagents

Efavirenz reference standard was purchased from Sigma Aldrich (Gillingham, Dorset, UK). Efavirenz 600mg tablets (Mylan Laboratories Limited, Maharashtra, India) were kindly supplied by the Princess Marina Hospital in Botswana.

Acetonitrile and HPLC water (both HPLC supra-gradient) were purchased from VWR International LTD (Lutterworth, Leicestershire UK).  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  and  $\text{KH}_2\text{PO}_4$  were purchased from Sigma Aldrich (Gillingham, Dorset, UK). The pH meter calibration standards of pH 4 and 7 were purchased from Mettler Toledo, Switzerland.

### **3.4.2 Chromatography conditions**

An Agilent HPLC 1200 series (Waldbronn, Germany) equipped with a degasser, binary pump, an auto sampler, column oven and diode array detector (DAD) was used. Separation was achieved on an Agilent ZORBAX SB C18 column (4.6 mm x 150mm x 3.5 $\mu\text{m}$ ) and detection at wavelength of 246 nm. The mobile phase consisted of phosphate buffer (25mM)-acetonitrile (53:47, v/v). The pH of mobile phase was adjusted to 7.5 using a diluted 2M solution of potassium hydroxide. The analysis was performed at room temperature with the flow rate maintained at 1.5 mLmin<sup>-1</sup> and injection volume of 50 $\mu\text{L}$ . The run time was initially set for 25 minutes but was adjusted to 15 minutes after establishing the RT of other alleged components in the nyaope. Analytical results were processed by Agilent Openlab Chromatography Data System/Chemstation/windows 7 Pro computer version A.02.01 (1.3.3).

### **3.4.3 Preparation of buffer solutions**

A 25mM phosphate buffer solution was prepared by dissolving 0.09g  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  and 0.34g  $\text{KH}_2\text{PO}_4$  in 100mL of HPLC grade water. The buffer was then mixed with acetonitrile at ratio 53:47. The resultant mixture was adjusted to pH 7.5 with dilute 2M KOH solution. The KOH solution was diluted 1:5 with deionised water before use. The pH meter was calibrated with a pH 4 and 7 calibrating standards before use.

The retention time for EFV was first established by preparing a standard solution of 1mg/mL EFV in methanol; three replicate injections of this solution were made and efavirenz was detected at 10.961minutes (figure 3.6).

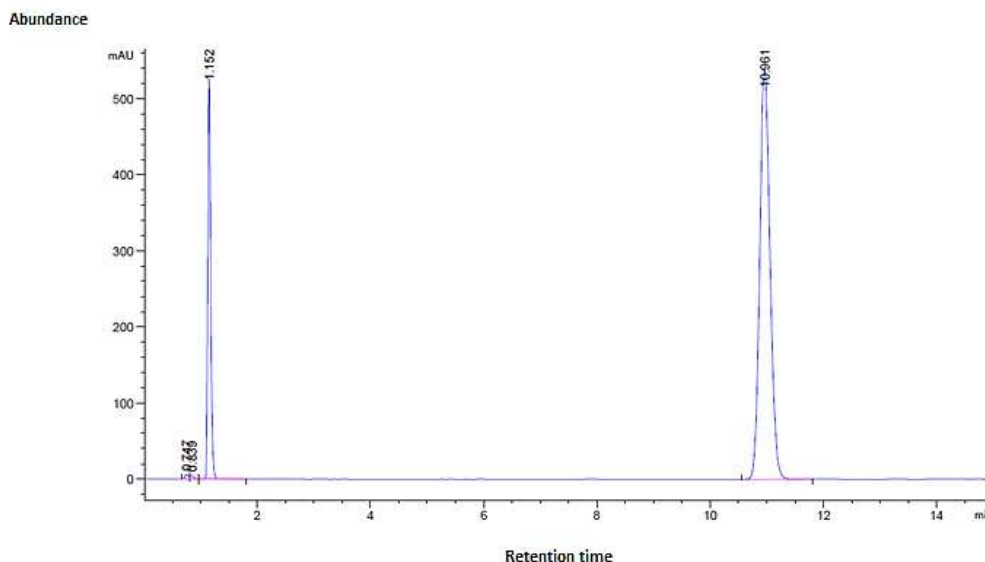


Figure 3.6 HPLC chromatogram showing caffeine just before 2 minutes and EFV at 10.961 minutes

### 3.5. HPLC Method revalidation parameters

#### 3.5.1 Preparation of standards

Stock solutions of efavirenz, 2-(4-Isobutylphenyl) propionic acid, caffeine and nevirapine were prepared by weighing the appropriate amount of each standard and dissolving it in the corresponding amount of mobile phase to yield concentrations of 1mg/mL each.

#### 3.5.2 Method Linearity

Standard stock solutions containing 1 mg/mL of efavirenz were prepared in triplicate. Aliquots of these solutions were diluted with methanol to make standard solutions of concentrations ranging from 100-500 $\mu$ g/mL. Data obtained from these were subjected to regression analysis using the least squares method to construct a calibration curve for the different concentrations versus peak area (table 3.10 and figure 3.7).

Table 3.10 Calibration data and %RSD results for the five HPLC calibration points

Replicate	100µg/mL	200µg/mL	300µg/mL	400µg/mL	500µg/mL
1	6856.489	12794.3	16694.6	24310.5	29996.8
2	6831.031	12778.5	16573.4	24488.4	30659.1
3	6819.571	12756.3	16569.4	24326.5	29928.7
Mean	6835.697	12776.37	16612.47	24375.13	30194.87
SD	18.89605	19.08961	71.15767	98.4175	403.4772
%RSD	0.276432	0.149413	0.428339	0.403762	1.336244

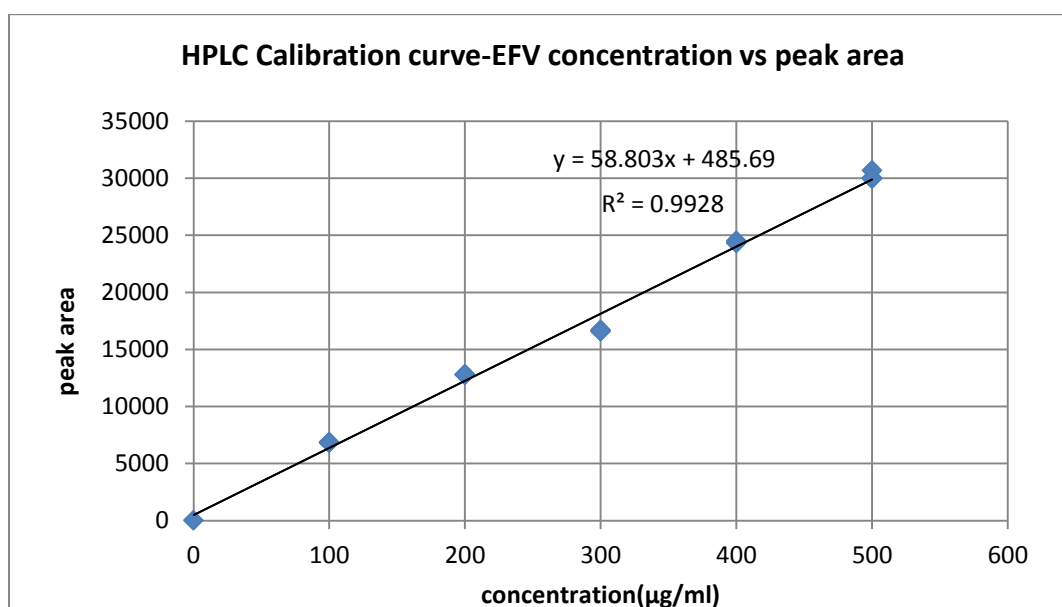


Figure 3.7 EFV calibration curve for the HPLC analysis

The correlation coefficient ( $R^2$ ) of efavirenz in methanol was found to be 0.993, confirming a linear relationship between the analyte and concentration. %RSD values for linearity concentrations (Table 3.4) ranged between 0.1-0.28%

### 3.5.3 Analytical Range

Based on the instrument calibration results, the method demonstrated linearity for EFV concentrations ranging between 100-600µg/mL.



### 3.5.4 Limit of Detection (LOD) and Limit of quantitation (LOQ)

The LOD and LOQ were derived from the standard deviation of the response and slope of the calibration curve and calculated using equations 3.2 and 3.3 (mentioned earlier in section 3.2.5). They were found to be 29 µg/mL and 88 µg/mL respectively.

### 3.5.5 Accuracy of the method

The accuracy of the HPLC method was determined by injecting known concentrations of certified reference material and comparing the measured value with the true value. Percentage analyte recoveries for the method (table 3.11) were found to be  $\geq 90\%$  (tables 3.10). %RSD for all analyte recoveries was found to be  $<5\%$ .

Table 3.11 HPLC method accuracy -analyte recoveries at different concentrations

Actual sample concentration (µg/mL)	Peak area	Calculated concentration (µg/mL)	Calculated accuracy (based on peak area) (%)
100	6856.5	105	105
100	6831.0	105	105
100	6819.6	105	105
200	12794.3	206	103
200	12778.5	206	103
200	12756.3	206	103
300	16694.6	273	91
300	16673.4	271	90
300	16569.4	270	90
400	24310.5	402	101
400	24488.4	405	101
400	24327.5	402	101
500	29996.8	499	100
500	30659.1	510	102
500	29928.4	498	100
		<b>Mean recovery</b>	<b>99.91%</b>
		<b>SD</b>	<b>5.23</b>
		<b>LOD</b>	<b>29</b>
		<b>LOQ</b>	<b>88</b>

**Table 3.12 Calculated %RSD for recovery at each calibration level**

<b>Replicate</b>	<b>100µg/mL</b>	<b>200µg/mL</b>	<b>300µg/mL</b>	<b>400µg/mL</b>	<b>500µg/mL</b>
1	105	103	91	101	100
2	103	103	90	101	102
3	105	103	90	101	100
<b>Mean</b>	<b>105</b>	<b>103</b>	<b>90</b>	<b>101</b>	<b>100</b>
<b>SD</b>	<b>0.32</b>	<b>0.16</b>	<b>0.40</b>	<b>0.42</b>	<b>1.37</b>
<b>%RSD</b>	<b>0.31</b>	<b>0.16</b>	<b>0.45</b>	<b>0.42</b>	<b>1.37</b>

### 3.5.6 Method precision

The repeatability factor was used to test for the method precision. To do this, 6 replicate injections of the 200µg/mL and 300µg/mL of EFV standard solutions into the HPLC. The mean, standard deviation and %relative standard deviation (%RSD) of peak areas across the six injections were calculated and evaluated. Results obtained (table 3.12) were found to be ≤5%, confirming excellent repeatability.

**Table 3.13 Results for HPLC method precision**

<b>Replicate injection</b>	<b>200µg/mL EFV standard</b>	<b>300µg/mL EFV standard</b>
1	12794	166945
2	12779	16573
3	12756	16569
4	12776	16612
5	12794	166956
6	12788	160382
<b>Mean</b>	<b>12781</b>	<b>16634</b>
<b>SD</b>	<b>14.45</b>	<b>57.12</b>
<b>%RSD</b>	<b>0.11</b>	<b>0.34</b>

### 3.5.7 Method selectivity

Method selectivity was investigated by performing 6 replicate injections of the laboratory standard into the instrument and calculating the %RSD values of each component across the resultant 6 peak areas. The standard was modified to contain only 1mg/mL EFV and 1mg/mL nevirapine. There was no need to include caffeine since it was used as an internal standard earlier (section 3.3.3), where it was found to elute at retention time different from that of EFV. The %RSD values of the two analytes were found to be within the acceptable level of  $\leq 5\%$  (figure 3.8) (table 3.13)

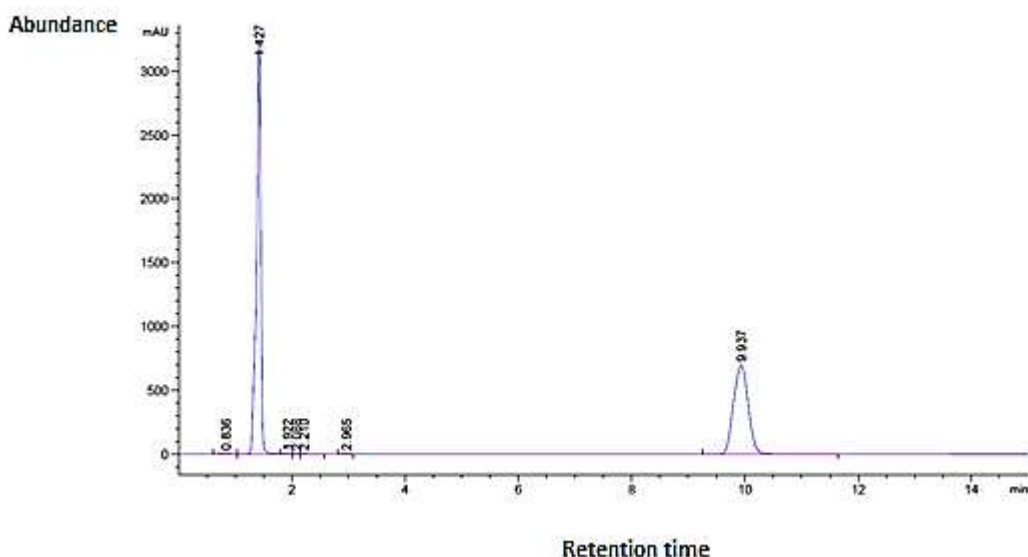


Figure 3.8 HPLC chromatogram showing nevirapine (1.427 minutes) and EFV (9.93 minutes)

The standard mixture was very well separated and the two components of the mixture did not elute at the same retention time, demonstrating good selectivity. Calculated %RSD values for peak areas for each sample were found to be  $\leq 5\%$  (table 3.14)

**Table 3.14 %RSD results for HPLC method selectivity**

<b>Replicate injection</b>	<b>1mg/mL EFV</b>	<b>1 m/mL nevirapine</b>
1	4734	16812
2	4728	16445
3	4745	16732
4	4617	15907
5	4724	16836
6	4712	16583
<b>Mean</b>	<b>4701</b>	<b>16552.56</b>
<b>SD</b>	<b>55.12</b>	<b>349.03</b>
<b>%RSD</b>	<b>1.19</b>	<b>2.11</b>

### **3.5.8 Analyte stability**

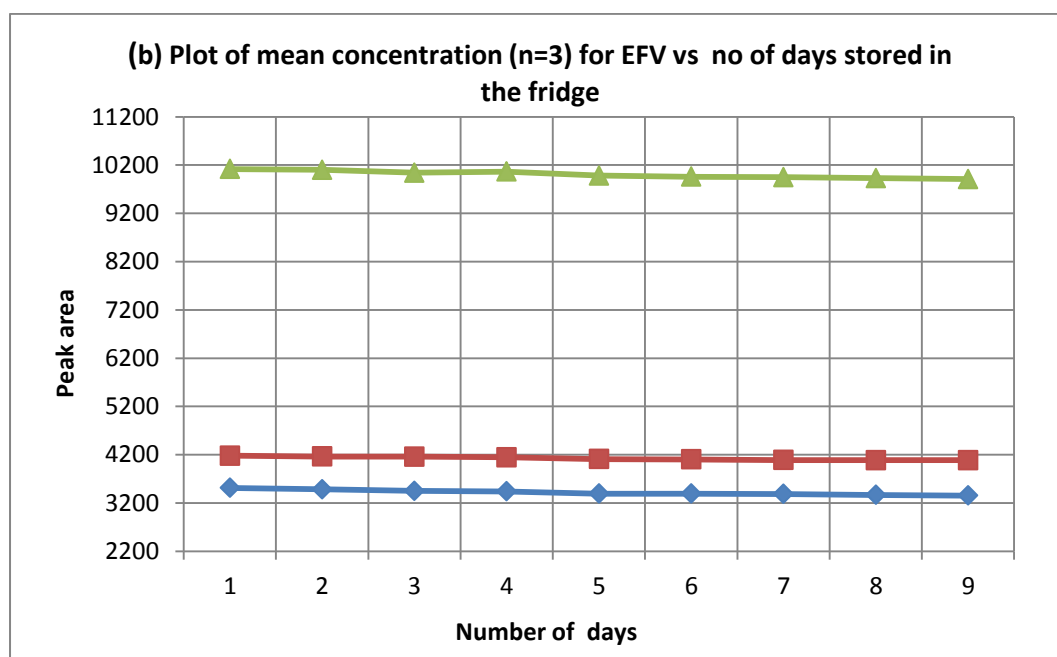
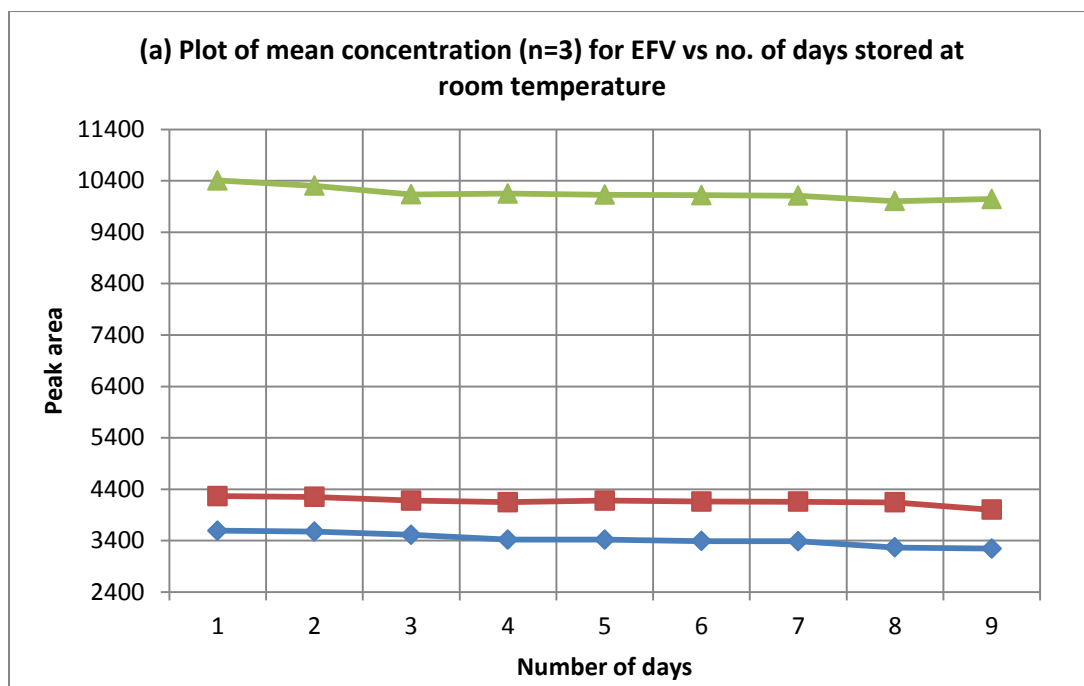
Analyte stability was tested by preparing two sets of EFV standard solutions at different concentrations of 200, 300 and 500 µg/mL and analysing them for 9 consecutive days. One set of solutions was kept in the fridge (2.5°C) while the other was kept at room temperature (19°C). Three replicate injections were made for each sample on daily basis. Daily mean peak areas at each concentration were calculated and used to generate graphs of peak area vs number of days (figures 3.9a and b) (table 3.15). Percentage relative standard deviations for each concentration were calculated. Even though all samples remained stable over the five day period, it was observed that those stored in the fridge were more stable. Calculated %RSDs values (table 3.15 and 3.16) for the three solutions at both temperatures were less than 5%.

**Table 3.15 %RSD results for analyte stability test at room temperature over 5 days**

Standard solution concentration		Average peak area (n=3)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Mean	SD	%RSD
500µg/mL	10119.9	10131	10152.6	10136.4	10303.4	<b>10168.66</b>	<b>76.239</b>	<b>0.74</b>
300µg/mL	4267.292	4250.081	4149.219	4178.729	4180.900	<b>4205.24</b>	<b>50.732</b>	<b>1.21</b>
200µg/ml	3595.359	3576.821	3513.994	3419.070	3418.934	<b>3504. 83</b>	<b>83.96</b>	<b>2.39</b>

**Table 3.16 %RSD results for samples stored in the fridge over 5 days**

Standard solution concentration		Average peak area (n=3)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Mean	SD	%RSD
500µg/mL	10119.9	10100.70	10066.50	10040.5	9981.8	<b>10061.88</b>	<b>54.23</b>	<b>0.54</b>
300µg/mL	4267.29	4250.08	4180.90	4149.22	4180.90	<b>4205.68</b>	<b>50.46</b>	<b>1.20</b>
200µg/mL	3576.82	3576.82	3513.99	3419.07	3418.93	<b>3501.13</b>	<b>79.24</b>	<b>2.26</b>



**Figure 3.9 Plot of EFV stability at a) room temperature and b) samples kept in the fridge for different concentrations (200, 300 and 500 µg/mL) of methanol**

\*Key: green = 500 µg/mL, red = 300 µg/mL, blue = 200 µg/mL

A summary of HPLC method validation results is presented in table 3.17

**Table 3.17 Summary of HPLC method validation results**

<b>Parameter</b>	<b>Results</b>
Linearity	100-500µg/mL
Cor. Co-efficient	0.99
Precision (%RSD): intraday repeatability	0.1-0.3%
Method specificity (%RSD) efavirenz with three other drugs	1-2%
Method accuracy	99 ±5%
LOD	30µg/mL
LOQ	88µg/mL
Analyte stability (%RSD) fridge (5 days) Room temperature (5 days)	1-4 % 4-5%

The various conditions tested for the High Performance Liquid Chromatography detection of the target analytes demonstrated validity of the method.

### **3.6 Generation of pyrolysed EFV samples in the absence and presence of other drug compounds.**

A number of different methods for abuse of EFV in combination with other drugs (cannabis, opium, heroin and methamphetamine) are known. All these involve heating the drug in the presence of EFV either indirectly by the application of a flame (for example using a flame to heat a spoon containing heroin or methamphetamine) or in combination with tobacco as a 'joint'.

As a consequence, a method for the generation of the pyrolysis products from EFV on its own and in combination with the other drugs commonly encountered was required in order to correctly simulate case samples. Two methods were needed, one involving the pyrolysis and combustion of a joint containing EFV and one where EFV was added to a sample of solid material (opium, heroin or methamphetamine) and heated.

### **3.6.1 The pyrolysis process**

Pyrolysis is a process involving molecular breakdown of larger molecules into smaller volatile molecules in the presence of heat without interaction with oxygen or any other oxidants. The process is necessary for almost all solids (or liquids) to burn [155, 156]. Every molecule within a substrate experiences some form of molecular vibration at any given temperature; the frequency at which it vibrates is directly proportional to its temperature [155]. During pyrolysis molecules are subjected to very high temperatures leading to very high molecular vibrations. At these high molecular vibrations, every molecule is stretched and shaken to such an extent that they start breaking down into smaller molecules [155]. Pyrolysis reactions causing molecular stretching vibrations have been reported [157].

Pyrolysis has also been described as a process which involves molecular fragmentation [157]; however this type of fragmentation is different from that of mass spectrometry as the resultant fragments are not charged.

When a substrate is freely burned, most of the pyrolysis products released by the substrate will be fuelling the flame. However, some will be adsorbed onto the substrate and may later be recovered during the extraction procedure [156].

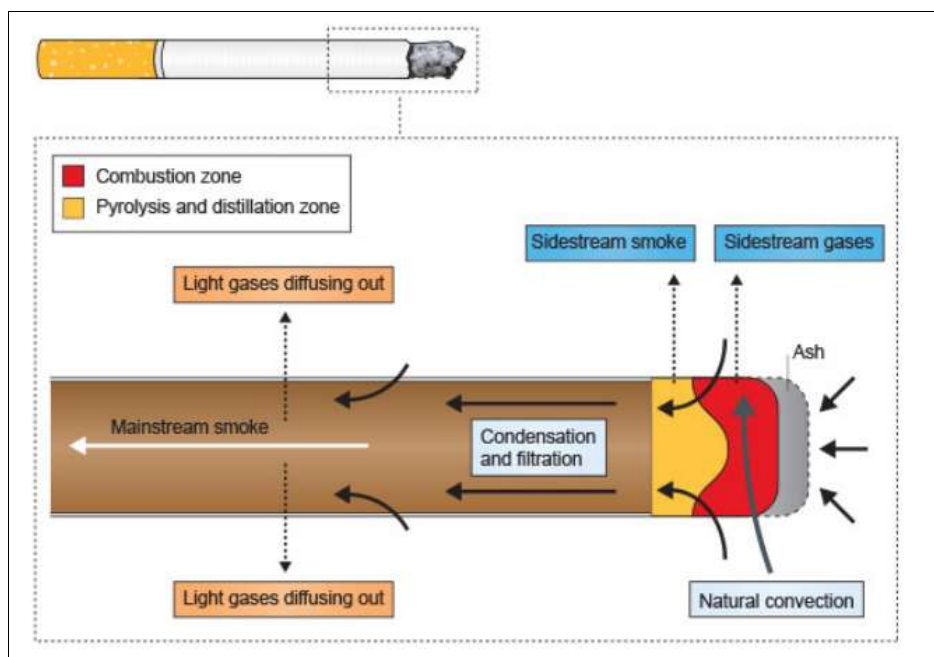
Examples of pyrolysis processes include those observed in polymers where decomposition occurs through main chain reactions of cross linking and chain scission or side chain /substitutions reactions of side chain elimination or cyclization [158].

### **3.6.2 Environment inside a smoked cigarette**

The environment inside a burning cigarette is hydrogen-rich and oxygen deficient and this allows the processes of combustion and pyrolysis/distillation to take place [159, 160, 161]. Several simultaneous chemical processes take place during the smoking of a cigarette, including pyrolysis and aerosol formation [156]. Combustion occurs around the tip of a cigarette and is mostly characterised by the presence of



an ember. The carbonised environment inside a cigarette consumes oxygen as soon as it is drawn into the cigarette during a 'puff', causing combustion to occur. Carbon monoxide, carbon dioxide and water together with the heat released during combustion sustain the burning process [157]. Immediately next to the combustion zone is a pyrolysis/distillation zone, which has relatively low oxygen levels and generally reaches temperatures of between 200°C and 600 °C. The majority of smoke products are generated in this region by a variety of mechanisms, essentially driven by the heat released from the preceding combustion zone [159]. During a 'puff', supersaturated vapour created by the smoke products are drawn down the tobacco rod as the mainstream of smoke and inhaled by the smoker. Taking a 'puff' in this way causes the air from outside the cigarette to enter through the paper line. This air dilutes and rapidly cools the generated mainstream smoke as it is drawn out of the pyrolysis zone [156, 161]. Cooling of the main stream smoke quickly brings vapours of less volatile compounds to their saturation point causing them to condense into ``a dense aerosol consisting of growing droplet particles of condensed pyrolysis products (smoke) which can be trapped and analysed [161].



**Figure 3.10 Schematic diagram for the combustion of a cigarette [161]**

### 3.6.3 Passive Headspace Extraction

When substances are heated, pyrolysis products are released from the sample matrix into the air above the surface of the heated material. If this were to take place inside a controlled environment, the released gases would be pre-concentrated onto the surface of a porous material and later desorbed with a suitable solvent for analysis. This is the basic principle of passive headspace sampling. The captured pyrolysis products (fig. 3.11) can be desorbed and then analysed using a GCMS or any other suitable instrumental method [101-106].

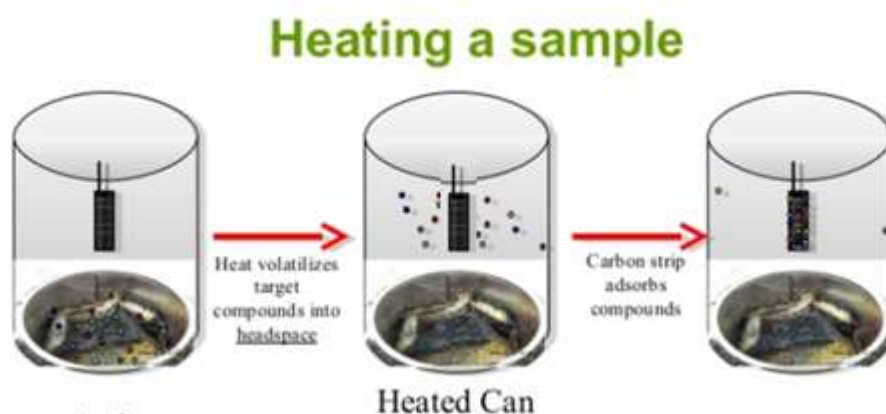


Figure 3.11 Schematic representation of the principle of passive headspace extraction technique [161]

The use of activated charcoal strips (ACS) as adsorbents has been known for many years [94, 95], however other adsorbents including DEFLEX and C-bags have also been used [96-99]. ACS has been viewed as an effective extraction method for fire debris by the American Society for Testing Materials (ASTM) in their ASTM-E1412-12 standard [94, 102] and, as a consequence, many forensic science laboratories have adopted them as adsorbents for fire debris analysis [95, 103, 104]. The effects of temperature (in the adsorption phase), strip size and adsorption time have all been investigated [94]. It has been recommended that temperatures above 90°C degrees are avoided when using ACS as degradation of substrates are likely to occur. The recommended adsorption time is between 12-16 hours at temperatures ranging between 60°C and 90°C [95, 104].

### **3.6.4 ACS experimental set up for capturing pyrolysis products.**

#### **3.6.4.1 Materials and methods**

EFV reference material was purchased from Sigma Aldrich, UK. 600mg EFV tablets were purchased from Pharma South, Gaborone Botswana. K type Thermocouple and cables, the Picolog data logger and software (Pico Technology, UK), ACS strips (3M), paper clips and small round magnets were all available within University of Dundee or purchased locally. Tin cans and lids were purchased from VWR Products, UK.

The methodology used was based on a design developed by Agu [163] which was adapted from the ASTM E1412 [32] guidelines. Activated carbon strips were placed inside a clean tin can lid and held in place using a paper clip and a magnet. This was to ensure that the ACS remained suspended above the material while it is being pyrolysed.

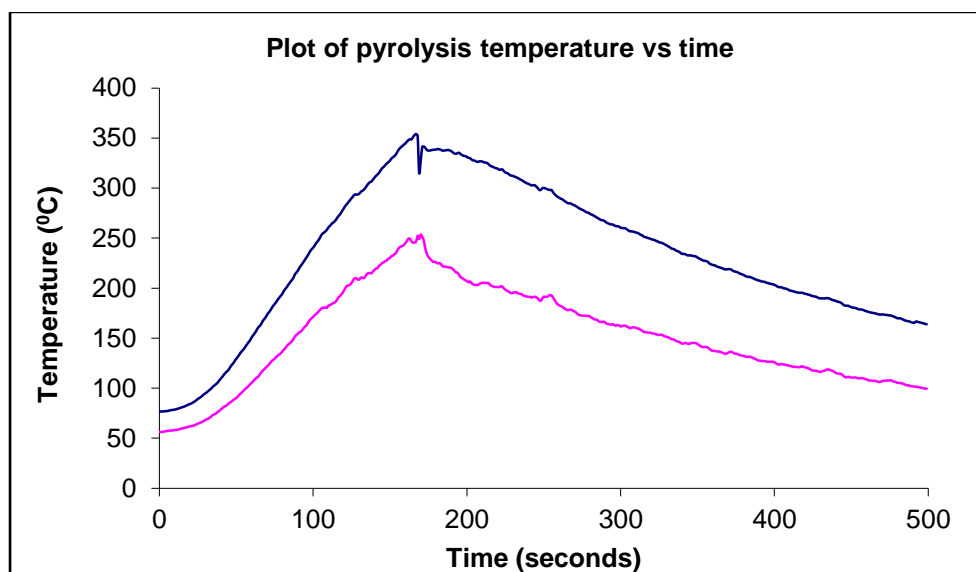
EFV tablets and any other samples which were not already in powder form were individually homogenised by crushing each into fine powder using a mortar and pestle. An amount corresponding to half the weight of the EFV tablet was used for each test. After weighing, the powder was heated to ensure pyrolysis occurred inside the tin can. The tin was capped during heating.

Once the desired pyrolysis temperature, (monitored using the thermocouple) had been reached the tin was removed from the heat source and the lid replaced with one onto which the ACS strips had been attached. The can was then incubated in an oven at 80°C for 16 hours. Following this, the ACS were removed and placed inside a GC vial containing 0.5mL of pentane. A 1mg/mL solution of caffeine standard was used as the internal standard. The heating temperatures were monitored and recorded using the picolog data recorder (figure 3.12)



**Figure 3.12** Experimental setup for the ACS pyrolysis experiment

Figure 3.13 shows a typical plot of temperature vs time generated from the picolog data during a pyrolysis experiment



**Figure 3.13** Plot of pyrolysis temperature vs time

### 3.6.5 Determination of the pyrolysis temperature for EFV.

In order to determine the optimum EFV Pyrolysis temperature range, a tablet of EFV was ground into a powder using a mortar and pestle and the powder (0.5g) was then heated to between 280°C and 450°C to cause thermal decomposition and the generation of pyrolysis products. The adsorption and desorption procedure described in section 3.5.4.1 was then followed.

#### 3.6.5.1 Pyrolysis products of EFV

Only one pyrolysis product of EFV was identified by GCMS analysis presenting at 11.79 minutes. A library search did not reveal the identity of the product (Product 1;  $m/z$  270, 252, 235, 210, 201, 185, 167). The mass spectrum of both EFV and product 1 are presented in figure 3.13a and figure 3.13 b.

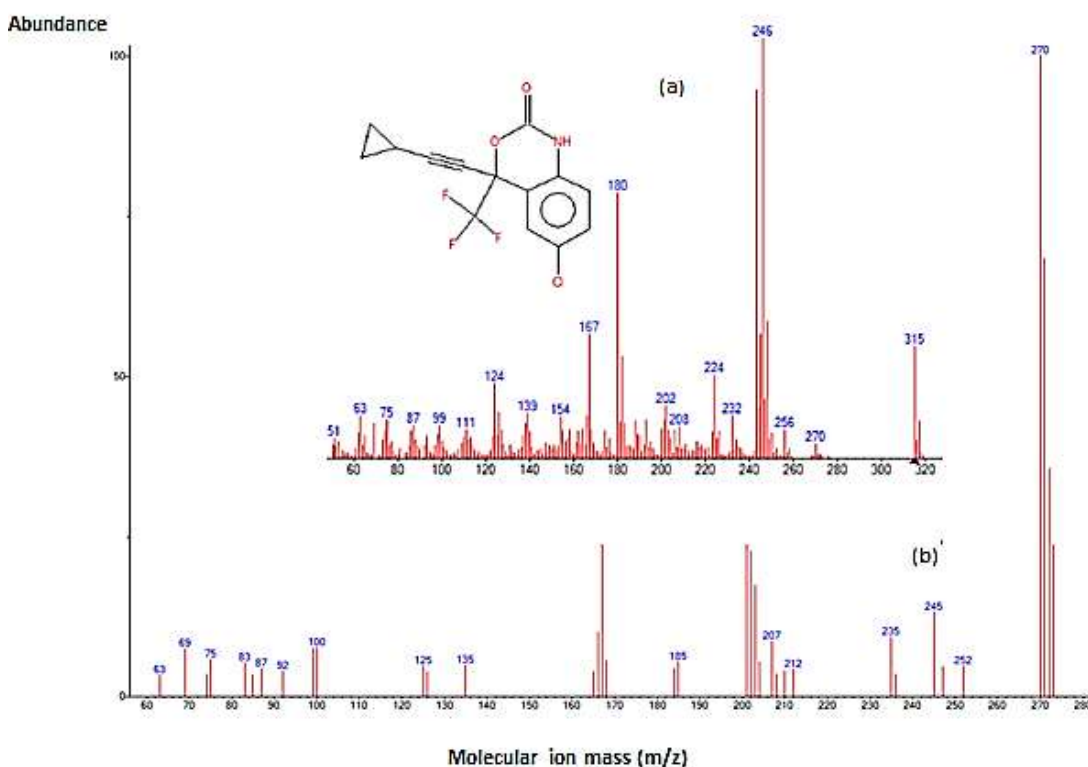


Figure 3.13 Mass spectra for (a) EFV and (b) EFV product 1

A tentative explanation of fragmentation suggests that the base peak of product 1 ( $m/z$  270) could have resulted from the loss of  $\text{CO}_2$  during ionisation;  $m/z$  201 could have resulted from the loss of  $\text{CHF}_3$  while  $m/z$  167 could have been due to loss of  $\text{C}_7\text{H}_{11}\text{OF}$ . Additional loss of  $\text{H}_2\text{O}$ ,  $\text{HCl}$  and  $\text{HF}$  resulted with ions  $m/z$  185, 210, 235 and 245.

#### **3.6.5.2 ACS Repeatability for EFV**

To assess repeatability, 0.5g EFV tablet powder was placed inside each of 6 tin cans and each can heated. All heating temperatures were based on the literature relating to pyrolysis and combustion of a cigarette (section 3.4.2) as this is a common form of how the material is consumed. The boiling and flash points ( $340^\circ\text{C}$  and  $159.8^\circ\text{C}$  respectively) of EFV were also taken into consideration. Each tin can contained 6 ACS strips suspended within the tin giving a total of  $n=36$  strips to assess the within can repeatability of the pyrolysis generation and extraction process.

Can 1 was heated to  $175^\circ\text{C}$ , can 2 to  $353^\circ\text{C}$ , can 3 at  $355^\circ\text{C}$ , can 4 at  $374^\circ\text{C}$  while can 5 and 6 were at  $378^\circ\text{C}$  and  $386^\circ\text{C}$  respectively. Each ACS strip within each can was removed, placed into a clean GC-MS sample vial and extracted by adding 0.5mL of the pentane. Six replicate injections were performed for each desorbing solution for each ACS strip. The peak areas and %RSDs were calculated across the six injections for each strip demonstrating excellent within tin repeatability for the production and extraction of the pyrolysis product and are presented in table 3.18 and figures 3.14 and 3.15.

**Table 3.18 EFV pyrolysis product peak area vs pyrolysis temperature**

ACS strip	Pyrolysis temperatures and average peak areas for each					
	175 <sup>o</sup> C	353 <sup>o</sup> C	355 <sup>o</sup> C	374 <sup>o</sup> C	378 <sup>o</sup> C	386 <sup>o</sup> C
1	7124520	1492438981	1423165240	958400994	883354598	2278462
2	7533409	1484723831	1434838802	958400994	883502169	2203772
3	7009784	1449681288	1491337756	958400994	888739443	2209059
4	7192589	1472867832	1445515087	958400994	894182859	2243810
5	7029019	1430724088	1483872522	959423895	889595852	2141265
6	7244520	1430724080	1473872522	959423895	88959852	2141265
<b>average</b>	<b>7177864</b>	<b>1466087204</b>	<b>1455745881</b>	<b>958605574</b>	<b>888161796</b>	<b>2215274</b>
<b>SD</b>	<b>212061</b>	<b>25531456</b>	<b>30253771</b>	<b>457455</b>	<b>4556681</b>	<b>51126</b>
<b>%RSD</b>	<b>2.95</b>	<b>1.74</b>	<b>2.08</b>	<b>0.05</b>	<b>0.51</b>	<b>2.31</b>

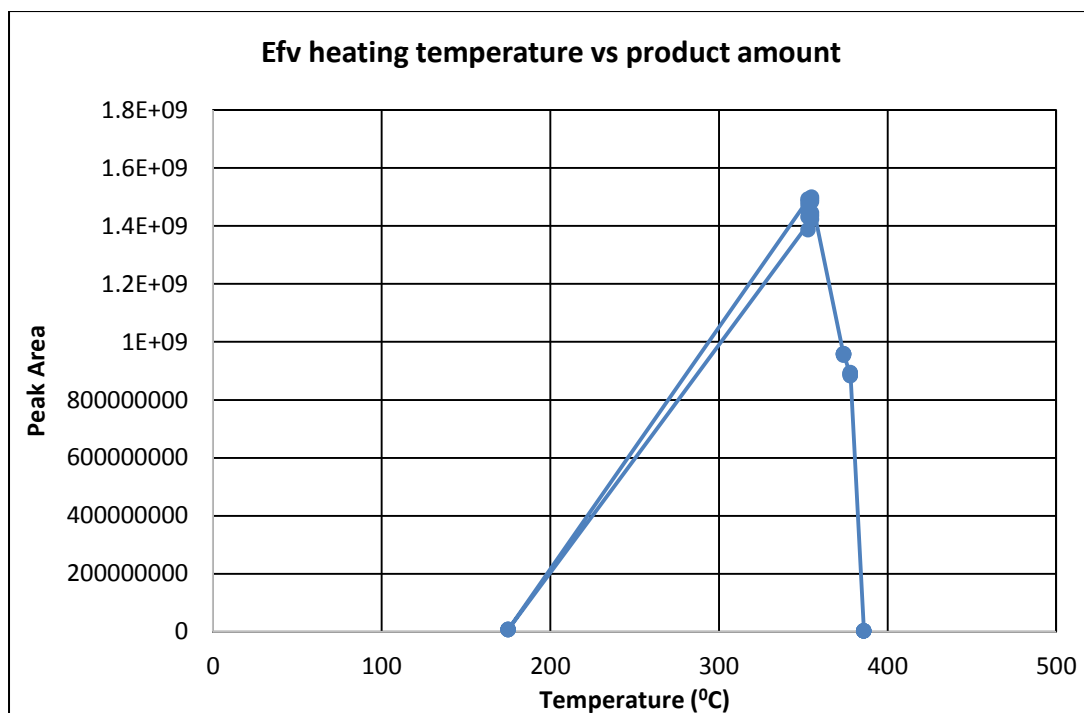


Figure 3.14 Plot of EFV pyrolysis temperature vs EFV pyrolysis product peak area.

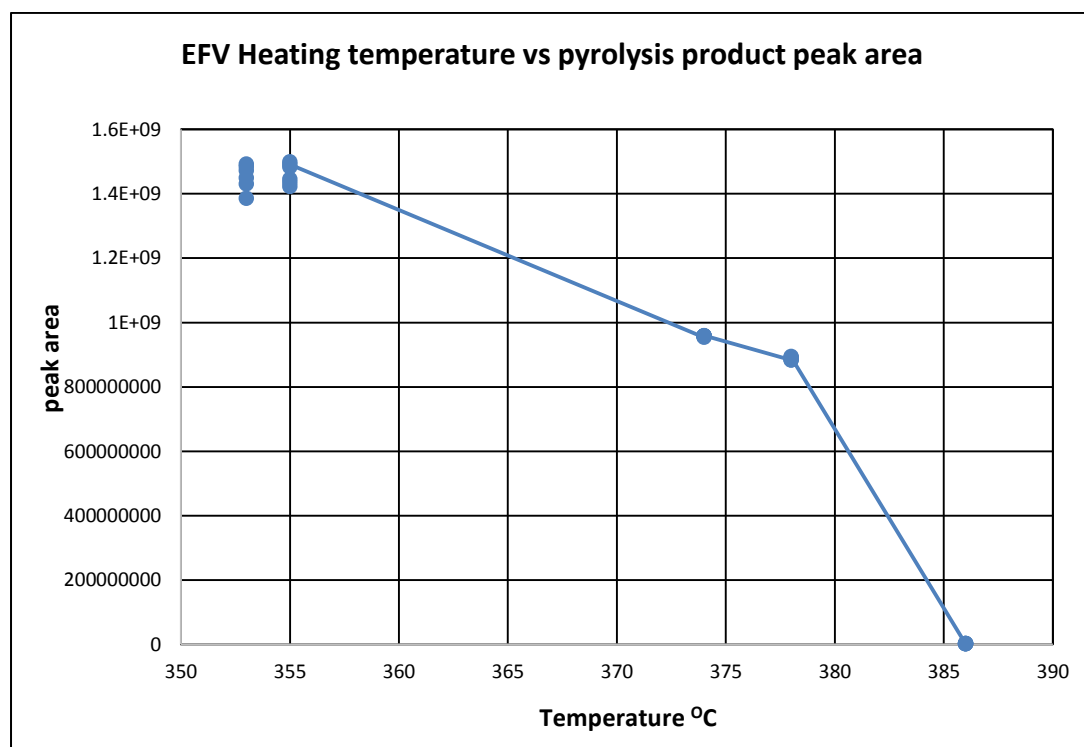


Figure 3.15 Expanded plot of EFV pyrolysis temperature vs pyrolysis product amount



The results obtained suggest that the largest peak areas representing the highest concentration of pyrolysis product were obtained between 353<sup>0</sup>C and 355<sup>0</sup>C. Heating either below or above these temperatures led, either to poor generation of the pyrolysis product or to degradation of the pyrolysis product. Based on these findings, it was decided that EFV samples and all other samples containing EFV would be heated between 353<sup>0</sup>C and 355<sup>0</sup>C.

### **3.7 Adaptation of GCMS method for the analysis of EFV**

The use of pentane as a desorbing agent for ACS was abandoned, because while it worked very well for the desorption of EFV on its own, it was not successful when used for other thermal decomposition products of drugs combined with EFV which would be expected to be encountered within the nyaope mixture. Pentane was initially the preferred solvent out of a number of those available for ACS desorption in the literature; because of its better desorption efficiency (meaning the ability to extract different classes of samples) and also that it is relatively safe to work with in the laboratory. Massey *et al* [98] 33, suggested that dichloromethane (DCM) was also an excellent solvent for ACS desorption, with high adsorption efficiency, particularly for alcohols and ketones which are commonly present in some illicit drug compounds [98].

Ultimately a combination of MeOH:DCM was required to ensure full sample desorption and two ratios were chosen (MeOH:DCM; 90:10 and 50:50) for further evaluation for GCMS analysis. A mini GCMS validation of both solvents was carried out to establish linearity, product stability, recovery, LOD and LOQ for each of the desorption solvent.

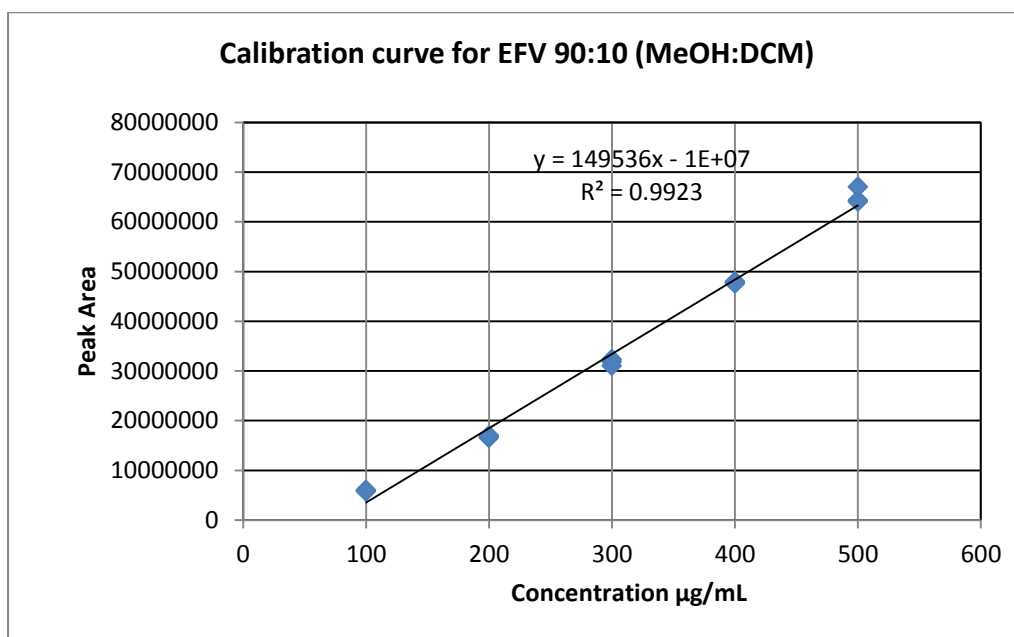
#### **3.7.1 GCMS mini validation for EFV using MeOH: DCM 90:10 solvent**

Five calibration standard solutions of 100 – 500µg/mL were prepared in the same manner as previously described. Three replicate injections of each solution were performed. Data obtained from these (Table 3.19) was subjected to regression

analysis using the least squares method to draw a calibration curve (figure 3.16). Correlation coefficient ( $R^2$ ) of efavirenz was found to be 0.992.

**Table 3.19 GCMS calibration data for EFV in MeOH-DCM 90:10**

Replicate	100µg/mL	200µg/mL	300µg/mL	400µg/mL	500µg/mL
1	5760354	16539579	32270348	47717734	64273757
2	5983685	16639589	31842348	47919989	64069912
3	5832825	16949576	31019867	47525841	67019434
<b>Mean</b>	<b>5858955</b>	<b>16709581</b>	<b>31710854</b>	<b>47721188</b>	<b>65121034</b>
<b>SD</b>	<b>113935.3</b>	<b>213772.3</b>	<b>635526.3</b>	<b>197096.7</b>	<b>1647219</b>
<b>%RSD</b>	<b>1.94</b>	<b>1.28</b>	<b>2.00</b>	<b>0.41</b>	<b>2.53</b>



**Figure 3.16 GC-MS calibration data of EFV for 90:10 MeOH-DCM**

Calculated %RSD values for linearity concentrations (table 3.18) ranged between 0.4-2.5% confirming that the method was linear between the concentrations of 100-500µg/mL. LOD and LOQ were found to be 44µg/mL and 133µg/mL

respectively. Accuracy of the method was found to be  $99 \pm 6$  % while the percentage analyte recovery was between 88- 106% (table 3.20 and 3.21).

**Table 3.20 Percentage recoveries, LOD and LOQ for extractions in MeOH-DCM 90:10**

Actual conc. ( $\mu\text{g/mL}$ )	Peak area	Calculated concentration ( $\mu\text{g/mL}$ )	Calculated % recovery
100	5760354	105	105
100	5983685	107	107
100	5832825	106	105
200	16539579	177	89
200	16639589	178	89
200	16949576	180	90
300	32270348	283	94
300	31842348	280	93
300	31019867	274	91
400	47717734	386	96
400	47919989	387	97
400	47525841	385	96
500	64273757	497	99
500	64069912	495	99
500	67019434	515	103
		<b>mean recovery</b>	<b>97</b>
		<b>SD</b>	<b>6</b>
		<b>LOD</b>	<b>44</b>
		<b>LOQ</b>	<b>134</b>

**Table 3.21 %RSD for analyte recovery extracted in MeOH-DCM 90:10**

% analyte recovery	CONC.( $\mu\text{g/mL}$ )				
	100	200	300	400	500
	105	89	94	96	99
	107	89	93	94	99
	106	90	91	96	103
<b>Mean</b>	106	89	93	97	100
<b>SD</b>	0.76	0.71	1.42	0.33	2.20
<b>%RSD</b>	<b>0.7</b>	<b>0.8</b>	<b>1.5</b>	<b>0.3</b>	<b>2</b>

### **3.7.2 Analyte stability for MeOH:DCM 90:10**

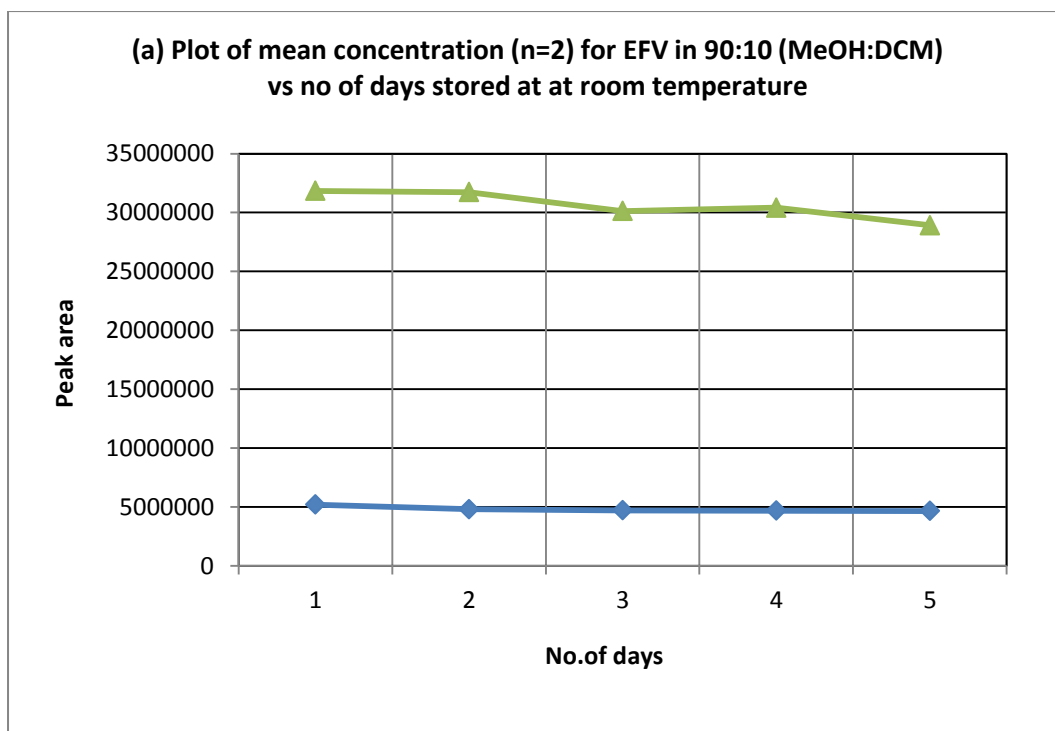
Samples for establishing stability were prepared like described earlier using EFV reference material of 100 and 300 $\mu$ g/mL concentrations. Injections were also performed in duplicates and daily mean peak areas at each concentration and are presented in Tables 3.22, 3.23 and figures 3.17a and b. Relative standard deviations for each concentration were also calculated. This experiment was performed for only 5 days because the calculated daily %RSD for samples stored at room temperature (table 3.21 and 3.22) remained within the set limit of  $\leq 5$  for only that period.

**Table 3.22 %RSD results analyte stability at room temperature over five days**

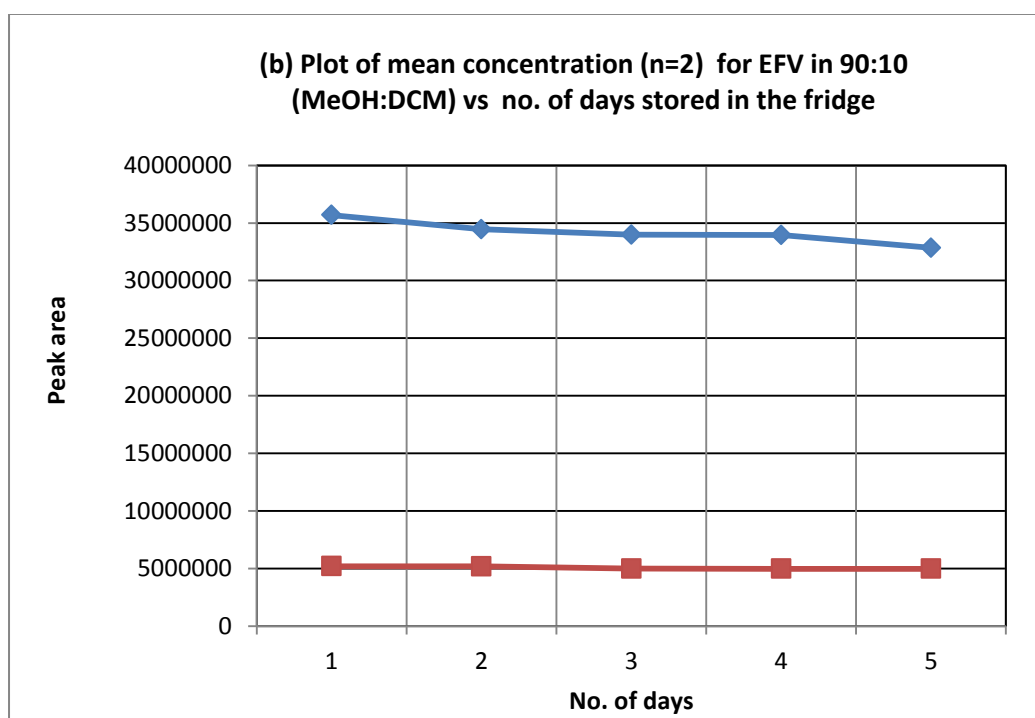
Standard solution concentration	Average peak area (n=2)							
	Day 1	Day 2	Day 3	Day 4	Day 5	Mean	SD	%RSD
<b>200µg/mL</b>	5206062	4809994	4711489	4684408	4654389	<b>4813268</b>	<b>227213.5</b>	<b>5</b>
<b>300µg/ml</b>	31840365	31738377	30133259	30410831	28917854	<b>30608137</b>	<b>1216277</b>	<b>4</b>

**Table 3.23 %RSD results for analyte stability in the fridge over five days**

Standard solution concentration	Average peak area (n=2)							
	Day 1	Day 2	Day 3	Day 4	Day 5	Mean	SD	%RSD
<b>200µg/mL</b>	5206061	5192987	4997791	4985532	4981497	<b>5072774</b>	<b>115954.4</b>	<b>2</b>
<b>300µg/mL</b>	35691125	34459171	33398453	33953559	32834294	<b>34184537</b>	<b>1032199</b>	<b>3</b>



\*Key: green = 300µg/mL, blue = 100µg/mL



**Figure 3.17 Plot of EFV stability at (a) room temperature and (b) fridge samples for different concentrations (100 and 300µg/mL) in MeOH-DCM 90:10 v/v**

\*Key: red = 100µg/mL, blue = 300µg/mL

### 3.7.3 Establishing GCMS linearity using MeOH:DCM 50:50 v/v solvent

Five calibration standard solutions of 100 – 500µg/mL were prepared in the same manner as previously described. Three replicate injections of each solution were performed. Data obtained from these (table 3.24) were subjected to regression analysis using the least squares method to draw a calibration curve (figure 3.18). The correlation coefficient ( $R^2$ ) of efavirenz was found to be 0.99. Calculated %RSD values for linearity testing were between 0.4 -3%. The analytical range at which the method was linear was between 100-500µg/mL. LOD and LOQ were found to be 95 and 107µg/mL respectively. Obtained analyte percentage recoveries were between 95 -107µg/mL and are presented in Table 3.25.

**Table 3.24 %RSD results for HPLC calibration using the MeOH-DCM 50:50**

Replicate	100µg/mL	200µg/mL	300µg/mL	400µg/mL	500µg/mL
1	7277336	25939117	44800749	64206227	82090428
2	7275648	25638198	42772571	64857321	82425991
3	7212933	24926501	44924472	60844263	82722333
<b>Mean</b>	<b>7255306</b>	<b>25501272</b>	<b>44165931</b>	<b>63302604</b>	<b>82412917</b>
<b>SD</b>	<b>36705.51</b>	<b>520009</b>	<b>1208270</b>	<b>2153732</b>	<b>316155.3</b>
<b>%RSD</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>3</b>	<b>0.4</b>

**Table 3.25 %RSD for percentage recoveries using MeOH-DCM 50:50**

% found recovery	Concentration (µg/mL)				
	100	200	300	400	500
	96	101	99	99	98
	96	99	103	102	99
	95	107	99	102	100
<b>Total</b>	287	307	301	302	298
<b>mean</b>	<b>95.66</b>	<b>102.19</b>	<b>100.18</b>	<b>101</b>	<b>99.25</b>
<b>SD</b>	<b>0.50</b>	<b>4.63</b>	<b>2.42</b>	<b>1.28</b>	<b>0.87</b>
<b>%RSD</b>	<b>0.5</b>	<b>5</b>	<b>2</b>	<b>1</b>	<b>1</b>

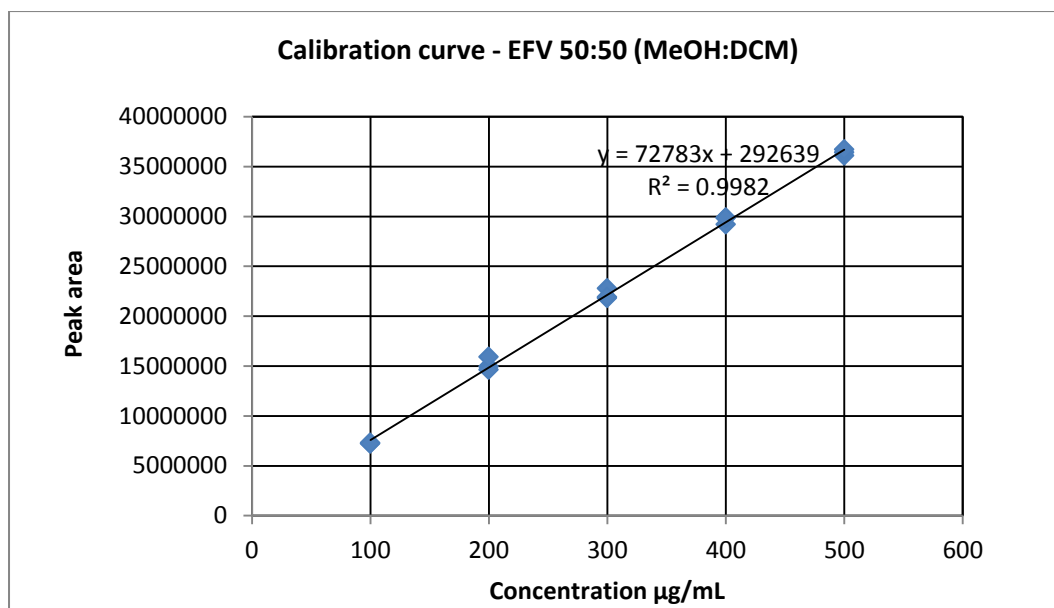


Figure 3.18 EFV calibration curve using 50:50 MeOH-DCM v/v

#### 3.7.4 Analyte stability for the MeOH-DCM 50:50 solvent

In order to be consistent with the stability experiment for the other solvent, this experiment was also performed across 5 days; however the test was stopped after only 4 days for room temperature samples because the analyte stability proved to be very unstable. Samples for establishing stability were prepared as previously described using EFV reference material. Injections were performed in triplicate. Daily mean peak areas at each concentration were calculated and used to generate graphs of peak area vs number of days (figure 3.19a and b). Relative standard deviations for each concentration were calculated. Calculated %RSD for samples kept in the fridge (table 3.26) were found to be within the acceptable range of  $\leq 5$  while those at room temperature (table 3.27) were outside the acceptable level. Following this, %RSD values for samples stored at room temperature were recalculated for the first two days (table 3.28), and were found to be within the  $\leq 5\%$ .

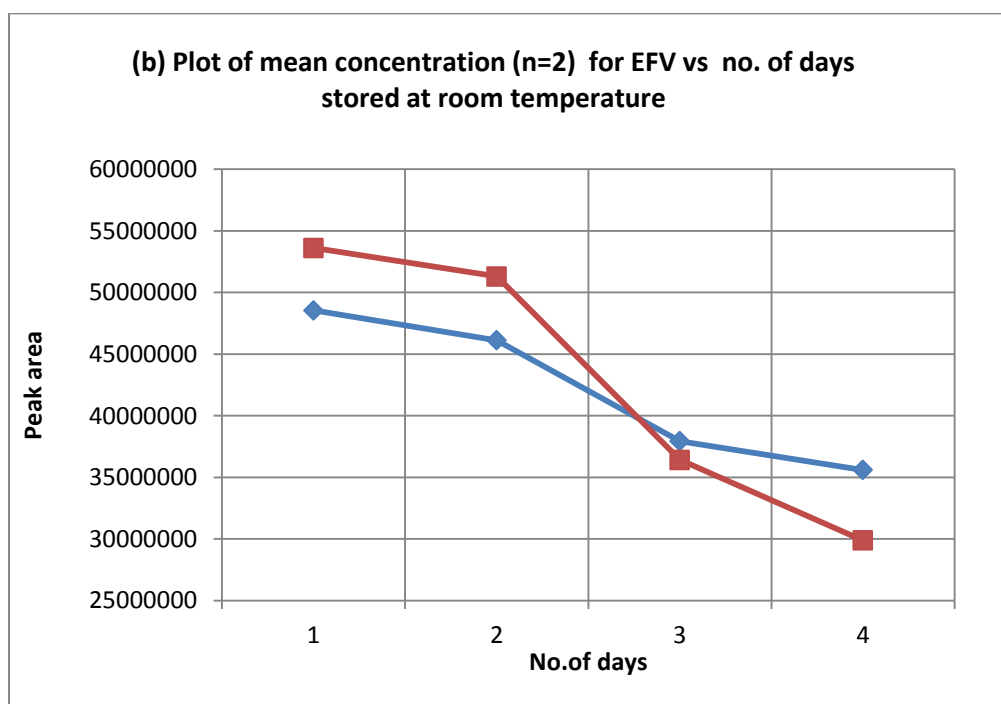
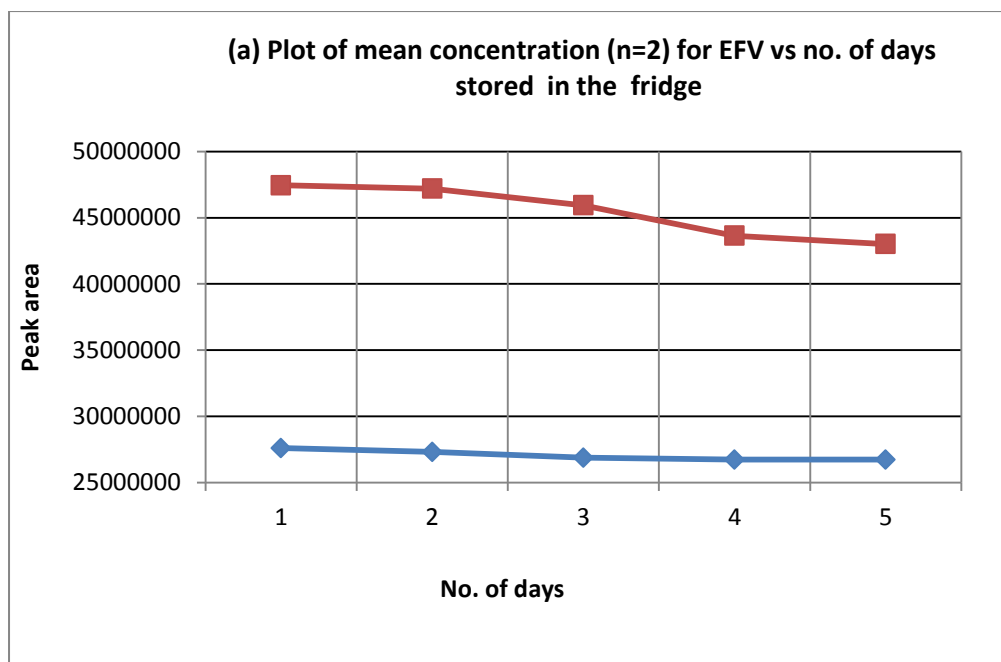


**Table 3.26 %RSD results for analyte stability test at room temperature over five days**

Standard solution concentration	Average peak area (n=2)							
	Day 1	Day 2	Day 3	Day 4	Day 5	Mean	SD	%RSD
<b>200µg/mL</b>	27602172	273121064	26897992	26735798	26728859	<b>27132502</b>	<b>397497.7</b>	<b>1</b>
<b>300µg/mL</b>	47454950	471953328	45937491	43633490	43020113	<b>45592103</b>	<b>1800933</b>	<b>3</b>

**Table 3.27 %RSD results for analyte stability for fridge samples over five days**

Standard solution concentration	Average peak area (n=2)						
	Day 1	Day 2	Day 3	Day 4	Mean	SD	%RSD
<b>200µg/mL</b>	48549452	46113476	37930360	35598256	<b>42047886</b>	<b>6254362</b>	<b>15</b>
<b>300µg/mL</b>	53613194	51289760	36409333	29881667	<b>42798489</b>	<b>11499640</b>	<b>27</b>



**Figure 3.19 Plot of EFV stability at a) room temperature and b) fridge samples for different concentrations (200 and 300 µg/mL) in MeOH-DCM 50:50 v/v**

\*Key: red = 300 µg/mL, blue = 200 µg/mL

**Table 3.28 %RSD results for analyte stability for first two days at room temperature extracted in MeOH-DCM 50:50**

Day	200 µg/mL	300 µg/mL
1	48549452	53613194
2	46113476	51289760
mean	<b>47331464</b>	<b>52451477</b>
SD	<b>1722495</b>	<b>1642916</b>
%RSD	<b>4</b>	<b>3</b>

### 3.8 Determination of HPLC repeatability for the ACS method.

Six replicate injections were performed for each extracted strip; mean peak areas and %RSDs were calculated across the six injections for each strip. The repeatability of each ACS sample set (within each tin can) was demonstrated excellently by within tin repeatability, as confirmed by the %RSD results (table 3.28).

**Table 3.29 %RSD results for inter-can analytical repeatability**

Replicate	Mean peak area can 1	Mean peak area can 2
1	97.22958	153.71959
2	98.33775	158.543
3	98.8492	156.81042
4	101.04742	159.88846
5	99.75414	161.09944
6	97.43099	159.73824
Mean	<b>99</b>	<b>158</b>
SD	<b>1.45</b>	<b>2.67</b>
%RSD	<b>1</b>	<b>1</b>

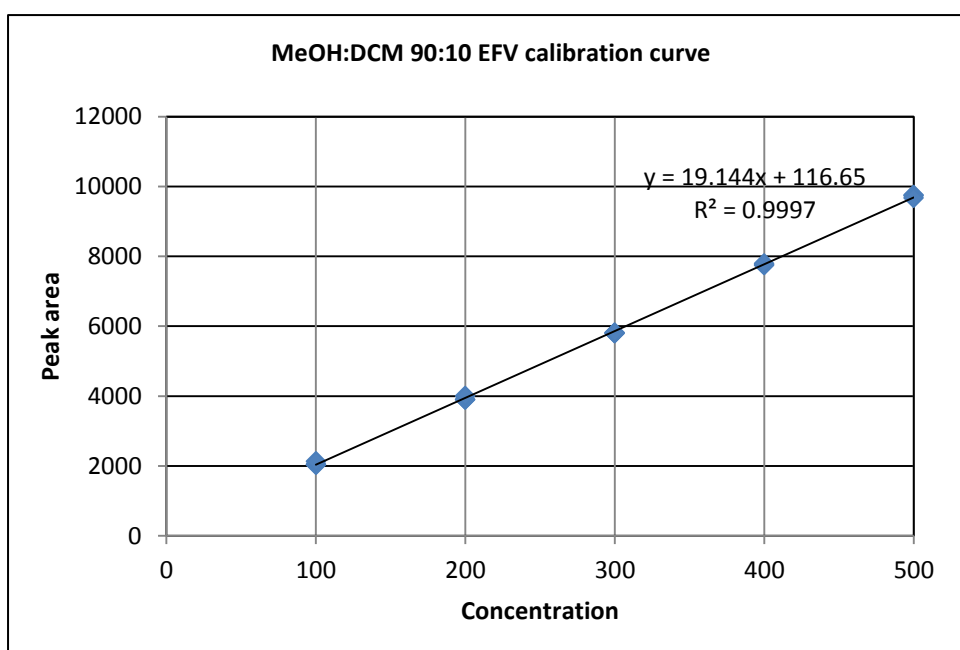
#### 3.8.1 Establishing the HPLC linearity using 90:10 solvents

Five calibration standard solutions of 100 – 500µg/mL EFV were prepared in the same manner as previously described. Three replicate injections of each solution were performed. Data obtained from these (table 3.29) were subjected to regression analysis using the least squares method to draw a calibration curve (figure 3.20). The correlation coefficient ( $R^2$ ) of efavirenz was found to be 0.99. Calculated %RSD values for linearity test were found to be between 0.1 -2.8.

The method is linear between the ranges of 100-500µg/mL. LOD and LOQ were found to be 9 and 28µg/mL respectively. Obtained analyte percentage recoveries were between 98 -105µg/mL (table 3.30 and 3.31).

**Table 3.29 %RSD for HPLC calibration using the MeOH-DCM 90:10 v/v/ solvent**

Replicate	100µg/mL	200µg/mL	300µg/mL	400µg/mL	500µg/mL
1	2028	3899	5800	7749	9753
2	2045	3899	5808	7756	9741
3	2131	4002	5812	7797	9672
<b>Mean</b>	<b>2067.88</b>	<b>3934</b>	<b>5807</b>	<b>7768</b>	<b>9722</b>
<b>SD</b>	<b>55.0247</b>	<b>59.414</b>	<b>5.810</b>	<b>25.92</b>	<b>43.91</b>
<b>%RSD</b>	<b>3</b>	<b>2</b>	<b>0.1</b>	<b>0.3</b>	<b>0.5</b>



**Figure 3.20 HPLC calibration curve for EFV in MeOH-DCM 90:10**

**Table 3.30 HPLC results for percentage recoveries, LOD and LOQ in the MeOH-DCM 90:10**

<b>Actual concentration(<math>\mu\text{g/mL}</math>)</b>	<b>Peak area</b>	<b>Calculated concentration (<math>\mu\text{g/mL}</math>)</b>	<b>%Recovery</b>
100	2027.7	100	100
100	2045.4	101	101
100	2130.6	105	105
200	3899.4	196	99
200	3899.4	198	99
200	4002.3	203	101
300	5800.3	297	99
300	5811.7	297	99
300	5807.9	297	99
400	7749.4	399	100
400	7756.4	399	100
400	7797.4	401	100
500	9753.5	503	101
500	9742.0	503	101
500	9672.3	499	100
		<b>Mean</b>	<b>100</b>
		<b>SD</b>	<b>1.60</b>
		<b>LOD</b>	<b>8.9</b>
		<b>LOQ</b>	<b>27.9</b>

**Table 3.31 %RSD results for recovery studies of the MeOH-DCM 90:10**

<b>Found conc. Replicate</b>	<b>Concentration (<math>\mu\text{g/mL}</math>)</b>				
	<b>100</b>	<b>200</b>	<b>300</b>	<b>400</b>	<b>500</b>
1	100	99	99	100	101
2	101	99	99	100	101
3	105	101	99	100	100
<b>Mean</b>	<b>102</b>	<b>100</b>	<b>99</b>	<b>100</b>	<b>100</b>
<b>SD</b>	<b>2.87</b>	<b>1.55</b>	<b>0.10</b>	<b>0.34</b>	<b>0.46</b>
<b>%RSD</b>	<b>3</b>	<b>2</b>	<b>0.1</b>	<b>0.3</b>	<b>0.5</b>

### 3.8.2 Establishing the HPLC linearity using MeOH:DCM 50:50 solvent

Five calibration standard solutions of 100 – 500 µg/mL were prepared as previously described. Upon inspection of the chromatograms, it was observed that use of the 50:50 (MeOH:DCM) solvent caused EFV to decompose. A double peak was observed at 8.973 and 9.491 minutes in all samples (figure 3.21). This was not the case when using the 90:10 MeOH:DCM mixture or the mobile phase.

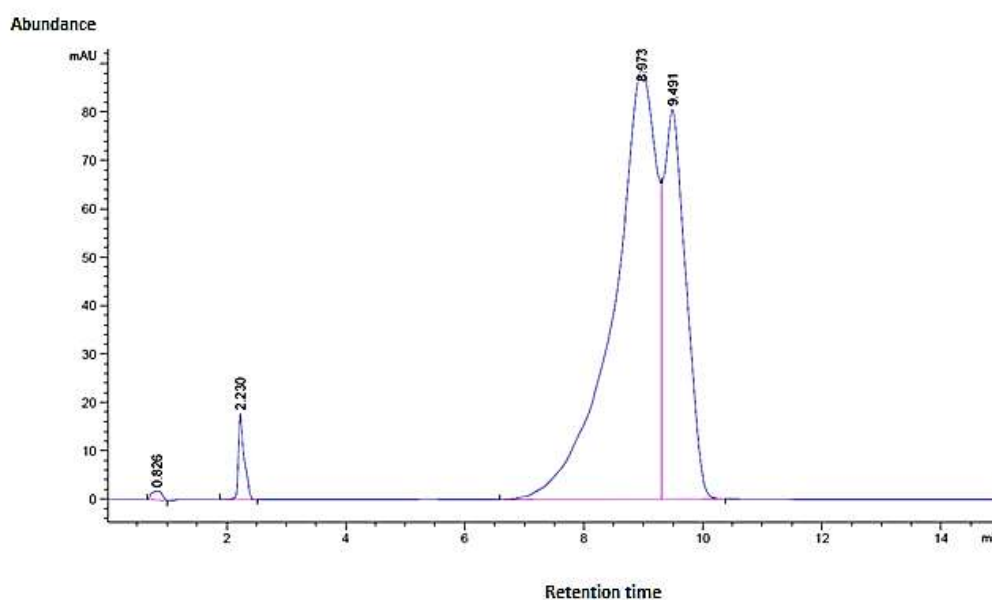


Figure 3.21 Chromatogram of 100 µg/mL EFV in 50:50 MeOH-DCM

A summary of ACS method validation results for both techniques is presented in table 3.32 below

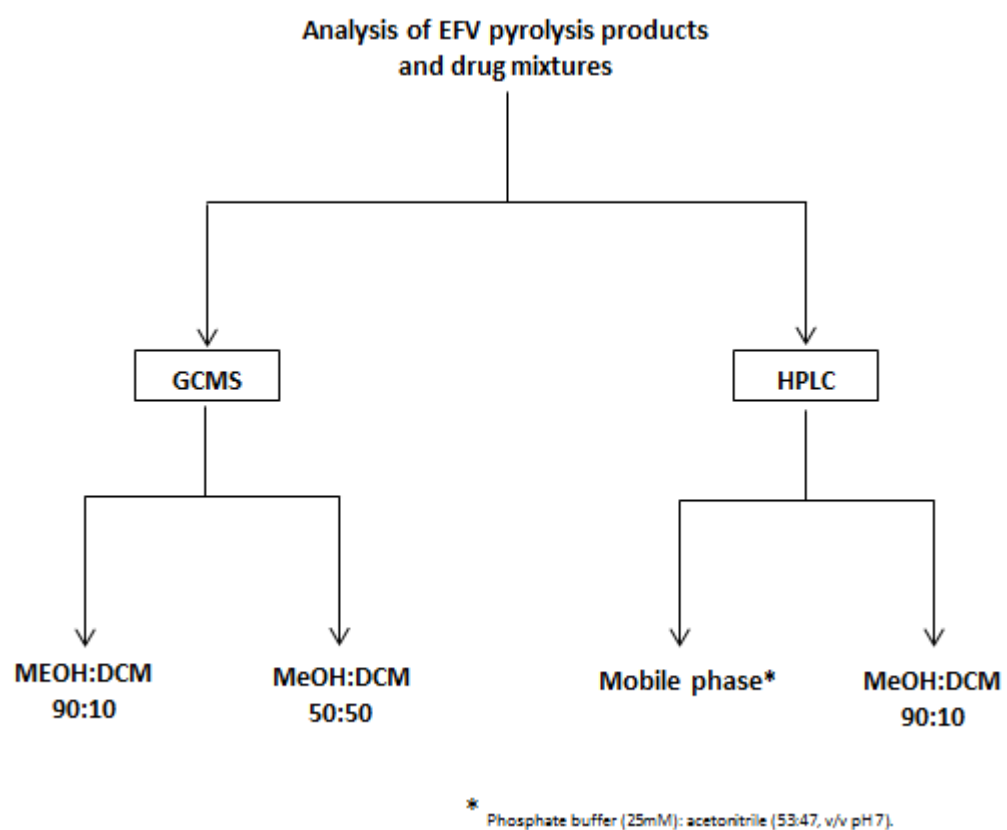
**Table 3.32 Summary of validation results for the GCMS and HPLC ACS method**

<b>Parameter</b>	<b>GCMS MeOH-DCM 90:10</b>	<b>HPLC MeOH-DCM 90:10</b>	<b>GCMS MeOH-DCM 50:50</b>
Linearity	100 - 500µg/mL	100 - 500µg/mL	100 - 500µg/mL
Cor.co-efficient	0.99	0.99	0.99
Precision (%RSD):repeatability	0.4-3%	0.1-3%	0.4-3%
Selectivity (%RSD) Laboratory test mix	1-2%	1±0.2%	1-2%
Method accuracy	99±6%	100±2%	99±1%
LOD	44µg/mL	8.9µg/mL	95µg/mL
LOQ	133µg/mL	28µg/mL	107µg/mL
Analyte stability (% srd):			
Fridge (5 days)	2-3%	n/a	1-3%
Room temp. (2 days)	4-5%	n/a	3-4%

The various conditions tested for the GCMS and HPLC detection of the target analytes in both solvents demonstrated validity of the method

### **3.9 Conclusions.**

A validated approach to the generation of pyrolysis products of the target analyte, efavirenz, and its analysis by both GCMS and HPLC was undertaken. As a result of this, the analytical methodology and workflows for the analysis of the simulated nyaope mixture involving EFV and a variety of target illicit drugs were structured and are presented in figure 3.22.



**Figure 3.22 Solvent strategies for the extraction of pyrolysis products of EFV and other drug mixtures**



## **CHAPTER 4: EFFECTS OF EFAVIRENZ ON THE SYNTHETIC DRUGS ALLEGEDLY USED IN THE NYAOPE MIXTURE.**

### **4.1 Introduction**

As previously discussed, the South African street drug nyaope, is allegedly made of a cocktail of many illegal substances ranging from heroin, cannabis, methamphetamine, opium to pain killers, where in each case the material also includes anti-retroviral drugs. The anti-retro viral drug, efavirenz, is believed to be the main ingredient in each case as it is thought to prolong the effects of other narcotic drugs in the mixture. The tablets are reportedly crushed into the mixture for smoking. There are various ways of smoking the nyaope; some people prefer to prepare joints while others prefer inhaling its vapours after heating over a flame on a piece of aluminium foil. The latter is a replica of a method developed in the 1950's by heroin smokers known as chasing the dragon [164].

In this chapter, the pyrolysis products of EFV in its mixture with methamphetamine, amphetamine and heroin were investigated. The experiment was carried out by first heating each drug on its own followed by heating a mixture of each with EFV and comparing the two analytical results. All pyrolysis products were trapped with activated carbon strips and analysed using both the GCMS and HPLC.

The reasons for the presence of impurities in illicit drugs which are clandestinely manufactured vary; impurities may be generated as by-product during drug manufacture, they may already be present in starting materials, reagents and/or solvents and may be carried over unchanged to the final product; or they may arise from reactions of original impurities in starting materials [165]. The relative amount of impurities in methamphetamine for instance, may show large variations, attributed to the exact nature of the starting materials, the synthetic route, actual manufacturing conditions used by the 'cook', the cutting agent added, storage conditions and methods of distribution [165]. Route specific impurities are those which, when present in an illicit substance, indicate the use of a specific synthetic pathway [165]. Studies of pyrolysis products of different drugs including

methamphetamine, amphetamine, heroin, opium and cannabis, exist [71, 81-87]. According to these studies, the word 'pyrolysis products' can mean either:

- (i) Impurities arising from synthetic pathways or from elsewhere (chemicals or other sources), for example benzaldehyde and phenyl-2-propanone as starting materials for amphetamine type stimulant drugs.
- (ii) Main pyrolysis products. These may arise from the breakdown of the parent drug or breakdown of intermediate products from synthetic routes and are usually used to determine the synthetic methods/routes applied during the manufacturing, for example acetyl-morphine for heroin or N-formylmethamphetamine as intermediate product of methamphetamine synthesis.

Similar pyrolysis products were obtained during this study and a similar strategy was applied.

## **4.2. Experimental**

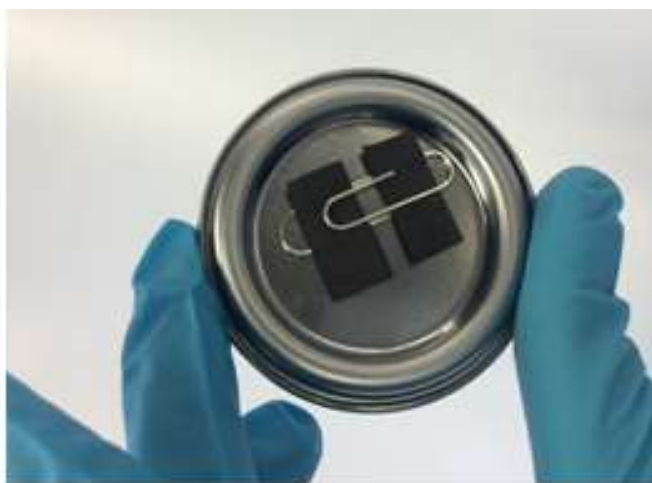
### **4.2.1 Chemicals and reagents**

Efavirenz (600mg) tablets were kindly supplied by the Princess Marina Hospital in Botswana. These were obtained from the supplies bought by the Botswana government for the Anti-retroviral therapy (ART) programme. The same type of EFV is also used by the South African government. Methamphetamine samples were in-house samples synthesised for research purposes. Opium, heroin and cannabis resin samples were available from completed forensic case samples. Acetonitrile (ACN), methanol (MeOH) and deionised water (all HPLC supra-gradient) were also purchased from VWR International LTD (Lutterworth, Leicestershire UK). Potassium hydroxide (KOH), Disodium hydrogen phosphate dodecahydrate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ) and Potassium phosphate monobasic ( $\text{KH}_2\text{PO}_4$ ) were all purchased from Sigma Aldrich (Gillingham, Dorset, UK). Dichloromethane (DCM) and methanol were analytical grade reagents and purchased from VWR International LTD (Lutterworth, Leicestershire UK). The pH meter calibration standards of pH 4 and 7 were purchased from Mettler Toledo, Switzerland. HPLC reference materials were purchased from Sigma Aldrich (Gillingham, Dorset, UK).

## 4.2.2 Instrumentation/Techniques

### 4.2.2.1 Pyrolysis procedure

All samples were heated using the apparatus described by Agu [163]. It consisted of a Picolog data logger and software (Pico Technology Ltd, Eaton Socon, United Kingdom), activated carbon strips (3M, United Kingdom), stainless steel paper clips and small round magnets (Amazon online), tin cans and lids (VWR Products, United Kingdom). The Sony laptop and hot plate were property of Dundee University. All individual drugs except EFV were heated at three different temperatures of 315°C, 354°C and 398°C. Samples in mixtures with EFV were heated to 354°C. The pyrolysis products were trapped using activated carbon strips. To ensure that the strips remained suspended above the sample headspace, they were first secured into a paper clip, put inside the tin can lid and a small magnet placed on top of the lid to hold them in place (figure 4.1).



**Figure 4.1 Presentation of a prepared tin can lid with the suspended ACS**

Heating temperatures were monitored and recorded using the picolog data recorder

#### 4.2.2.2 Pyrolysis product extraction procedure and ACS sampling strategy

For analysis with the GCMS, one strip (out of the four in a can) was extracted using 0.5mL methanol-dichloromethane (MeOH/DCM) 90:10 v/v and the other with 0.5mL MeOH/DCM 50:50v/v. The remaining two strips were analysed with the HPLC where one was extracted with the mobile phase and the other with 0.5mL MeOH/DCM (90:10 v/v). Six replicate injections were performed for each extract using each analytical instrument. Figure 4.2 summarises the sampling strategy.

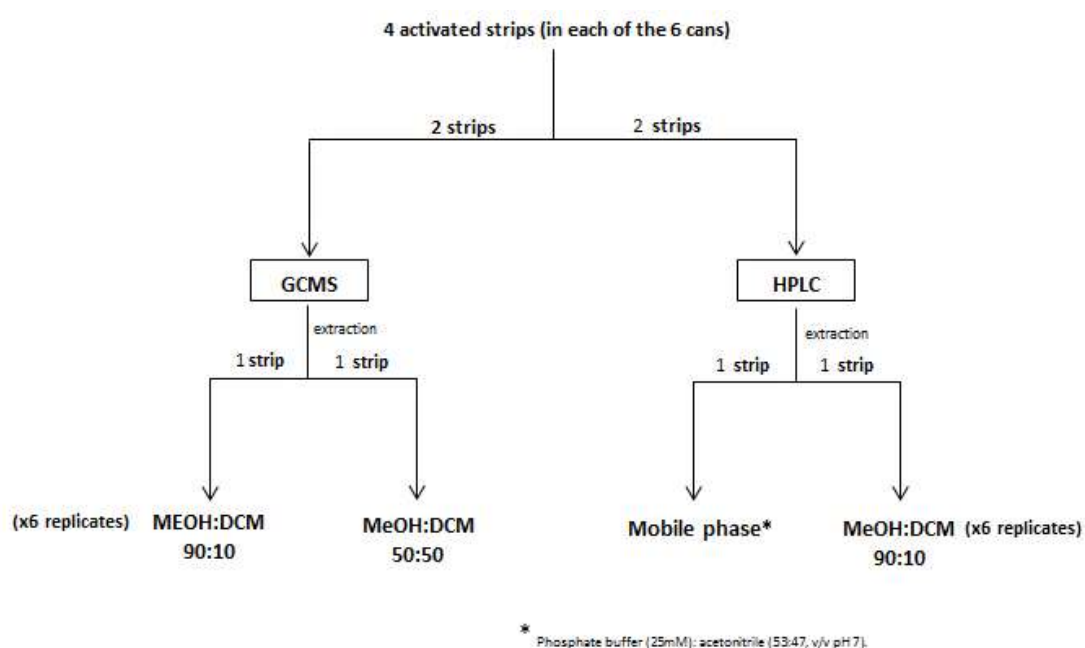


Figure 4.2 Overall ACS sampling strategy for each technique

#### 4.2.2.3 GCMS conditions

Pyrolysis products were identified using an Agilent 7820A gas chromatograph interfaced with an Agilent 5977E mass selective detector, operated under conditions described in Chapter 3.1.2. Compounds were identified using both the NIST 2014 mass spectrum database and data from gathered literature.

#### **4.2.2.4. HPLC conditions**

An Agilent HPLC 1200 series (Waldbronn, Germany) equipped with a degasser, binary pump, an auto sampler, column oven and diode array detector (DAD) was used for the analysis of all samples. Operating conditions and settings for the HPLC were set as described earlier in Chapter 3.2.3. The mobile phase was prepared and the pH adjusted as discussed in Chapter 3.2.4.

### **4.3 Isolation of pyrolysis products of EFV and analysis with GCMS**

Six replicate EFV samples were prepared following the protocol described in chapters 4.2.2.1 where EFV tablets (600mg) were first homogenised by crushing them into fine powder using a mortar and pestle. 0.5g of the tablet powder was then weighed into 6 separate tin cans, heated and pyrolysis products extracted and trapped using four ACS in each tin can. Following this, the analysis sampling strategy in Chapter 4.2.2.2 was applied where one of the four ACS strips was extracted with MeOH-DCM 90:10 and the second one with MeOH-DCM 50:50.

#### **4.3.1 Results and discussions**

Only one EFV pyrolysis product (figure 4.3) was revealed in all analysed samples. The product was detected at 11.09 minutes and presented a fragmentation pattern with main four molecular ions of  $m/z$  270, 201, 167, 245 and 235. The product was labelled as EFV pyrolysis product 1. The results were verified against those obtained from the EFV standard sample during the GCMS method validation.

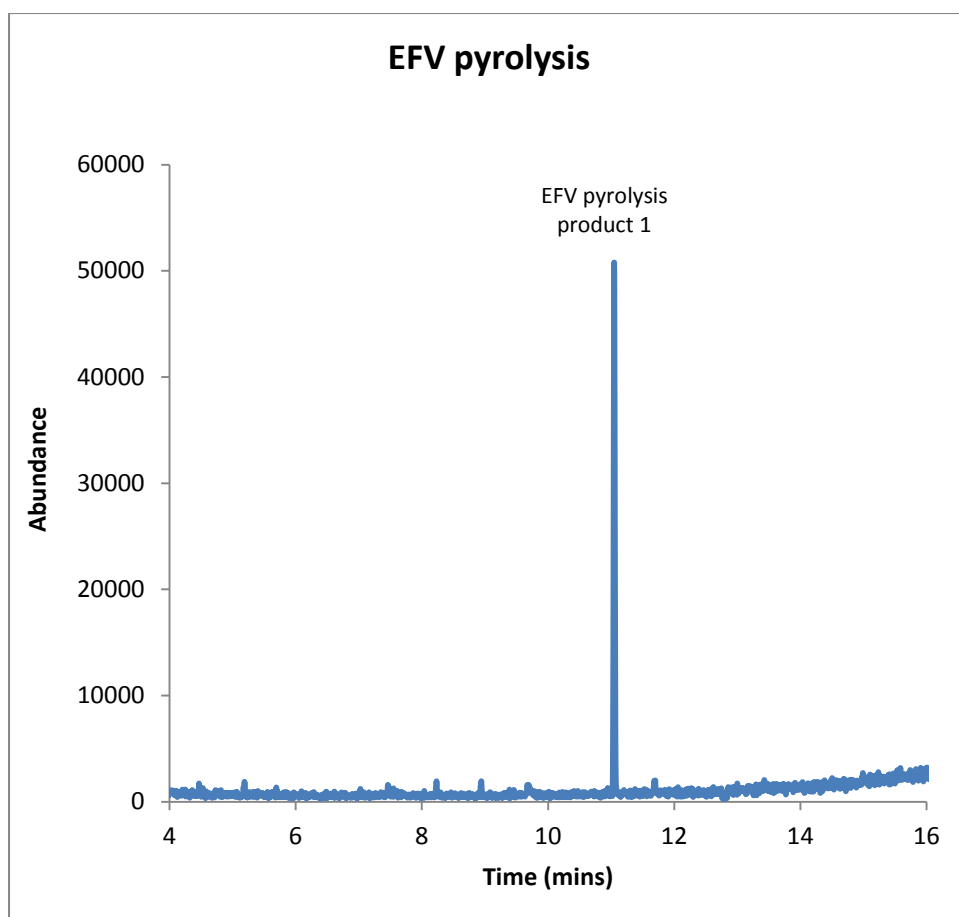


Figure 4.3 Chromatogram for EFV pyrolysis product 1

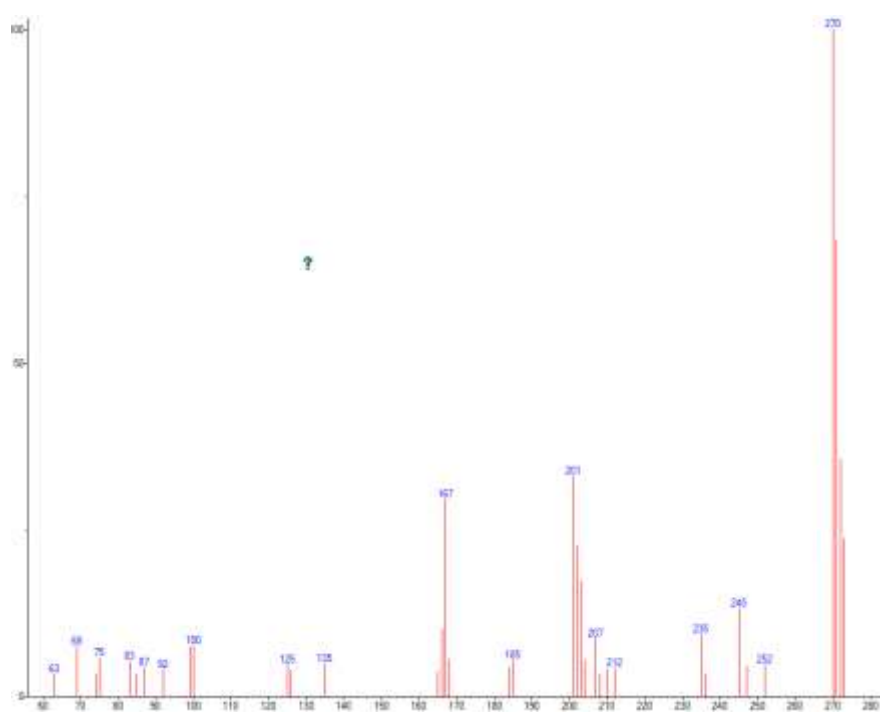


Figure 4.4 Mass spectrum of EFV pyrolysis product1

The mass spectrum of the pyrolysis product was compared with that of EFV and it was confirmed that only one molecular ion ( $m/z$  167) was common to both. A superimposed image of both spectra is shown in figure 4.5 and a proposed structure of EFV pyrolysis product 1 presented in figure 4.6.

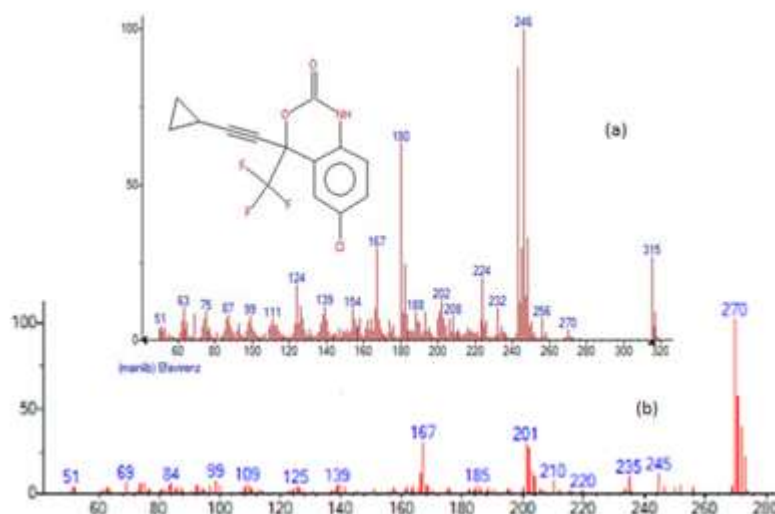


Figure 4.5 Superimposed mass spectral images of (a) EFV (b) EFV pyrolysis product 1

#### 4.4 Isolation of pyrolysis products of EFV and analysis with the HPLC

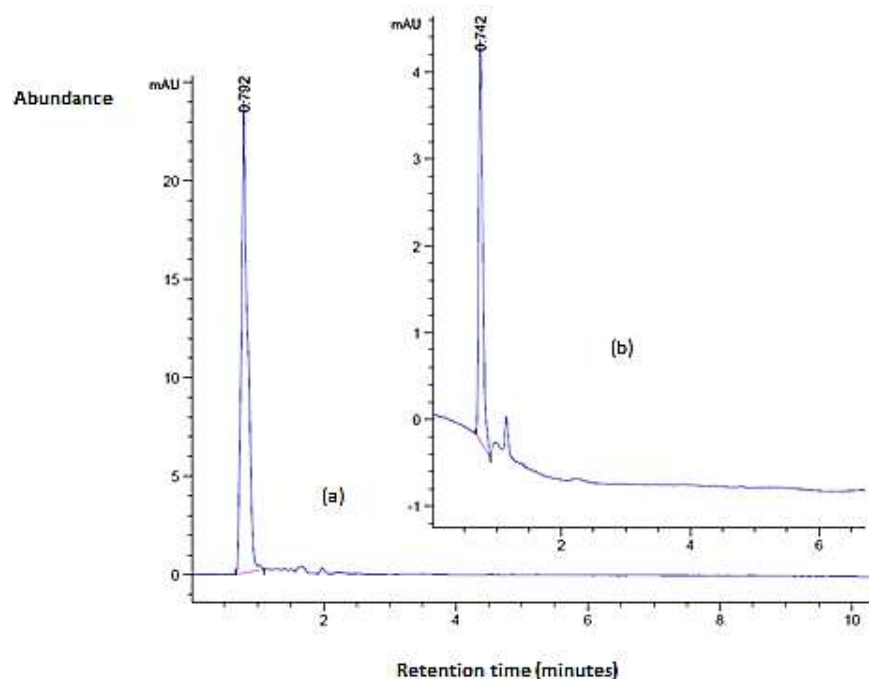
EFV samples used for the HPLC analysis were the two remaining ACS strips from the four prepared earlier in section 4.3. As per the project sampling strategy in section 4.2.2.2, one strip was extracted with the MeOH-DCM 90:10 while the other with the mobile phase. Negative control samples were prepared by running fresh ACS through the same sample preparation and analysis procedure. Reference materials were also analysed in the mobile phase and the MeO:DCM 90:10.

##### 4.4.1 Results and Discussions

##### 4.4.1.1 Results obtained from the pyrolysis of fresh ACS samples

The results obtained from the pyrolysis of fresh ACS (figure 4.7a and b) showed the presence of one product in each solvent; these were detected at 0.792 and 0.742

minutes for the mobile phase and MeOH:DCM 90:10 respectively. Analysis of both solvents also did not reveal the presence of any pyrolysis product, and as such the two products observed were considered to be artefacts from the activated carbon strips.



**Figure 4.7 HPLC results showing ACS artefacts observed in the ( a) mobile and (b) MeOH:DCM 90:10**

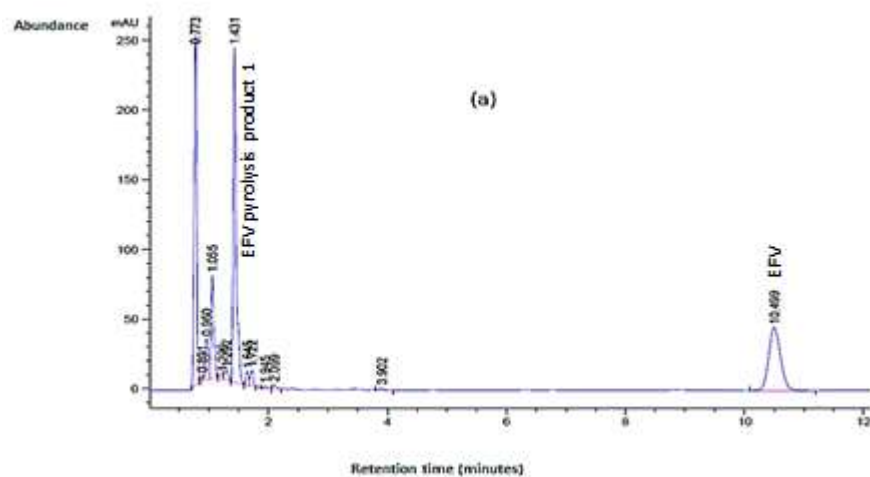
#### **4.4.1.2 Results for the analysis of pyrolysis of EFV using the HPLC**

The results obtained (figure 4.8 (a) for extraction of the analyte using the mobile phase and 4.8 (b) for extraction of the analyte using MeOH-DCM 90:10 v/v) revealed the presence of only EFV and its pyrolysis product 1. The pyrolysis product was detected at 1.431 minutes in the mobile phase and at 1.423 minutes using the MeOH-DCM 90:10 v/v. These results were verified against those obtained from the EFV standard sample during the HPLC method validation (table 4.1)



**Table 4.1 Results for the HPLC reference materials in the mobile phase and MeOH:DCM 90:10**

Reference material	Retention time (minutes) with mobile phase	Retention time (minutes) with MeOH:DCM 90:10
Benzaldehyde	1.26	1.67
Allylbenzene	1.60	2.40
1,2 Dimethyl -3- phenylaziridine,trans	2.39	2.52
P2P	2.45	3.38
Methamphetamine	3.26	3.97
di-methyl amphetamine	3.81	12.29
N-formyl methamphetamine	11.46	12.61
N-acetylamphetamine	11.74	12.02
Morphine	1.35	1.40
Meconin	1.72	1.91
Noscapine	2.85	2.75
Papaverine	8.45	7.90
Codeine	2.29	2.98
Thebaine	5.82	4.80
Cannabinol	5.43	5.43
Cannabidiol	3.28	3.28



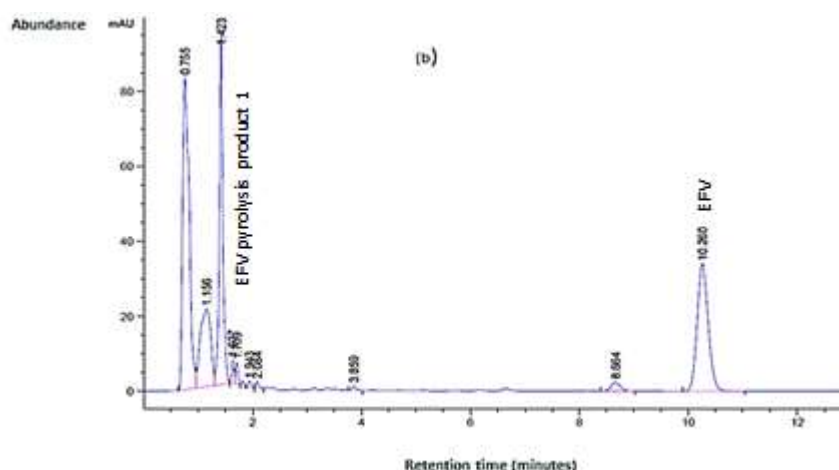


Figure 4.8 HPLC chromatogram for the pyrolysis of EFV in (a) the mobile phase and (b) MeOH-DCM 90:10

## 4.5 Isolation of pyrolysis products of methamphetamine and analysis with the GCMS

### 4.5.1 Sample preparation

Six replicate methamphetamine samples were prepared following the protocol described in section 4.2.2.1. Methamphetamines were sourced in-house from samples previously prepared under known synthetic conditions. A mixed methamphetamine sample containing samples from more than one synthetic method was used to better simulate real life conditions. In this case samples prepared using the Leuckart, Emde] and Nagai synthetic routes [166-176] were within the mixed sample.

The methamphetamine samples were first homogenised by crushing them into fine powder using a mortar and pestle. 0.5g of the powdered material was weighed into 6 separate tin cans. Each tin can was covered with a lid, heated to the desired temperature and removed from the hot plate. The covering lid was replaced with the one with prepared CAS strips (section 4.2.2.1) and incubated for 16 hours at 80°C to trap the pyrolysis products. The products were trapped using four ACS in each of the six tin cans. Following this, the analysis sampling strategy in section

4.2.2.2 was applied. For GCMS analysis one of the four ACS strips was extracted with MeOH-DCM 90:10 and the other with MeOH-DCM 50:50.

#### **4.5.2 Results and discussions**

Results obtained from samples extracted with the two different solvents ratios are displayed in table 4.2. Typical chromatograms for pyrolysis products obtained from the pyrolysis of methamphetamine at 354<sup>0</sup>C (extracted using MeOH-DCM 90:10 and MeOH-DCM 50:50) is displayed in figure 4.9 and 4.10. Detected pyrolysis products in both cases have been reported as impurities from various chemicals and reactions used during the synthesis of methamphetamine [168, 169], and, given their survival of the pyrolysis process have the potential to be used to assist in the determination of methamphetamine synthetic routes. Four main pyrolysis products of methamphetamine are displayed in figure 4.11.

**Table 4.2 Pyrolysis products of methamphetamine extracted with the two MeOH-DCM solvent ratios at 3 different temperatures**

ACS extracting solvent	Pyrolysis Temperature (°C)		
	315	354	396
<b>MeOH:DCM 50:50</b>	N-Formylmethamphetamine N-Methylbenzamide trans 1,2 Dimethyl -3-phenylaziridine Allylbenzene Benzaldehyde	N-Formylmethamphitamine N-Methylbenzamide norephedrine Deoxyephedrine 1-Phenyl-1-Propane Trans 1,2-dimethyl-3-phenyl Aziridine 1-propenyl benzene Benzaldehyde	N-Formylmethamphitamine norephedrine Methoxyphenamine 1-Phenyl-2-Propanone trans 1,2-dimethyl-3-phenyl Aziridine 1-propenyl- benzene Cyclopropyl-benzene Benzaldehyde
<b>MeOH:DCM 90:10</b>	N-Methylbenzamide trans 1,2 Dimethyl -3-phenylaziridine Methamphetamine Amphetamine Cyclopropylbenzene Benzoic acid Allylbenzene Benzaldehyde Ephedrine N-acetylmethamphetamine di-methylamphetamine N-methylamphetamine	Methamphetamine 1-Phenyl-2-Propanone 1-Propenylbenzene Benzaldehyde cyclopropylbenzene N-acetylmethamphetamine di-methylamphetamine N-acetylmethamphetamine	Ephedrine Deoxyephedrine Ethyl phenyl ketone Trans 1,2-dimethyl-3-phenyl Aziridine 1-Propenylbenzene Benzaldehyde

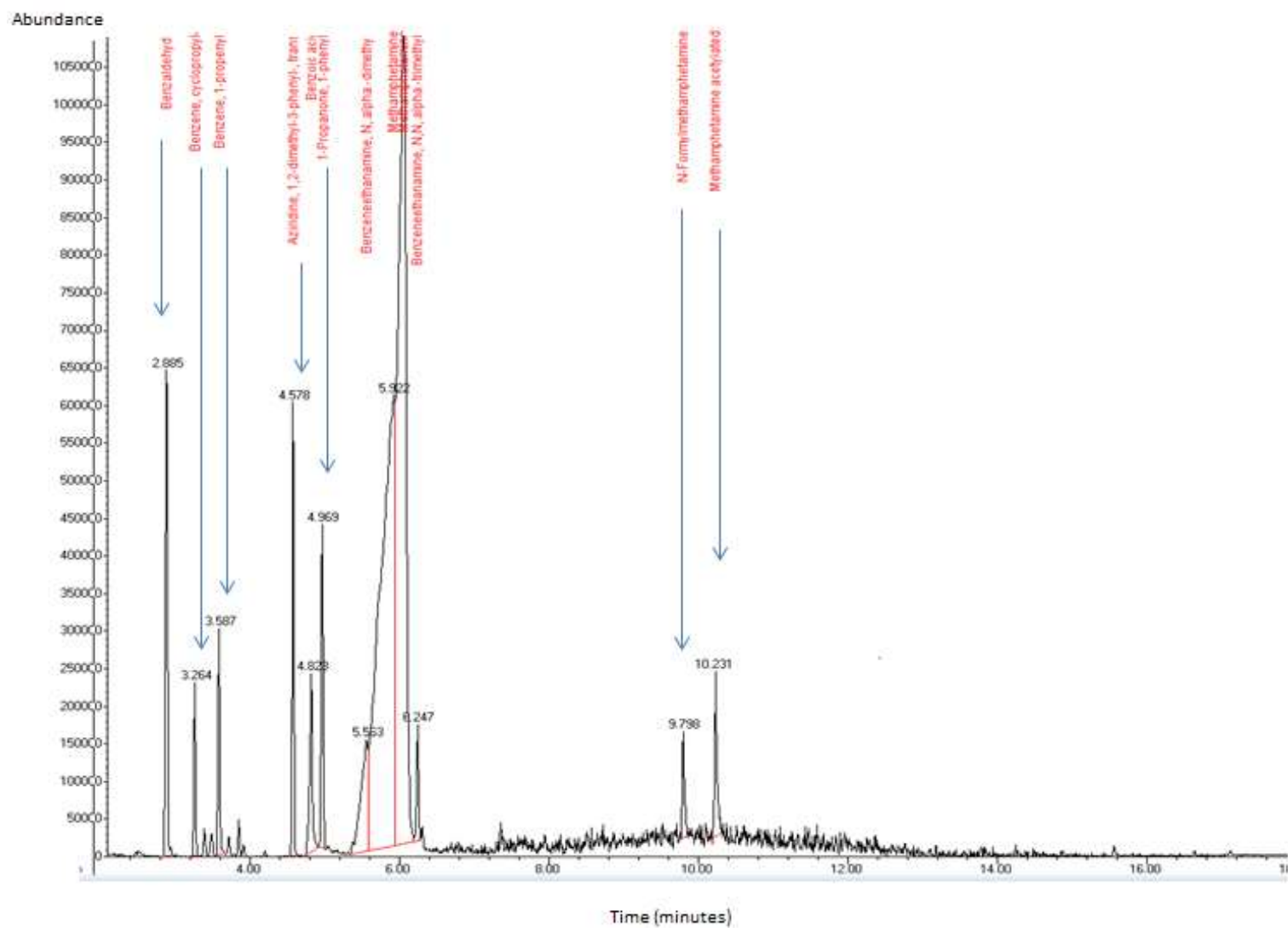


Figure 4.8 Chromatogram for pyrolysis products of methamphetamine extracted in MeOH-DCM 90:10 at 354°C

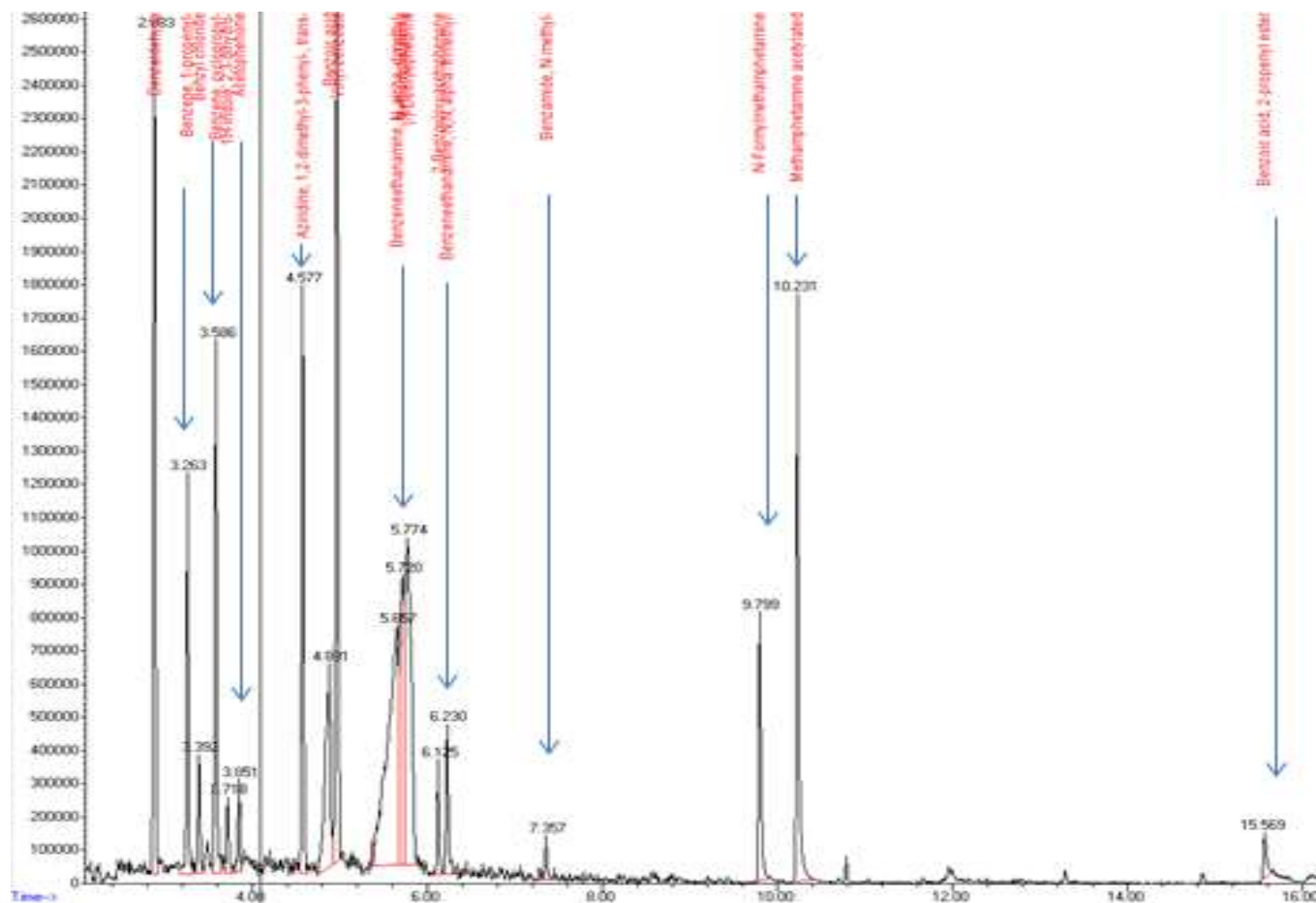


Figure 4.10 Chromatogram for the pyrolysis products of methamphetamine extracted in MeOH-DCM 50:50 at 3540C

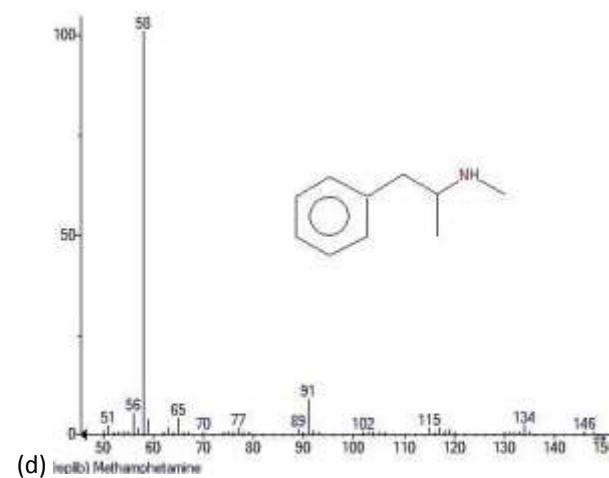
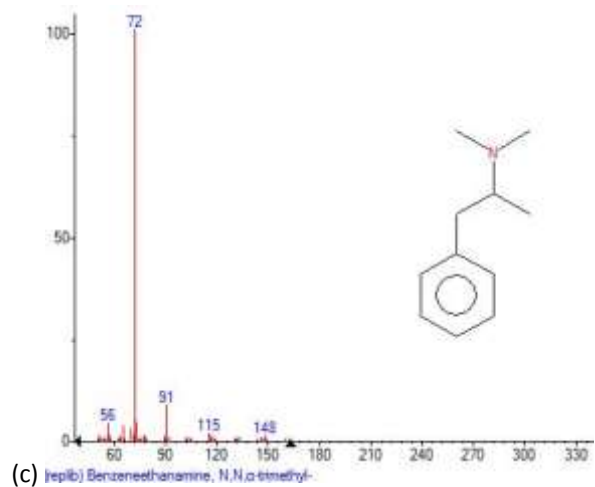
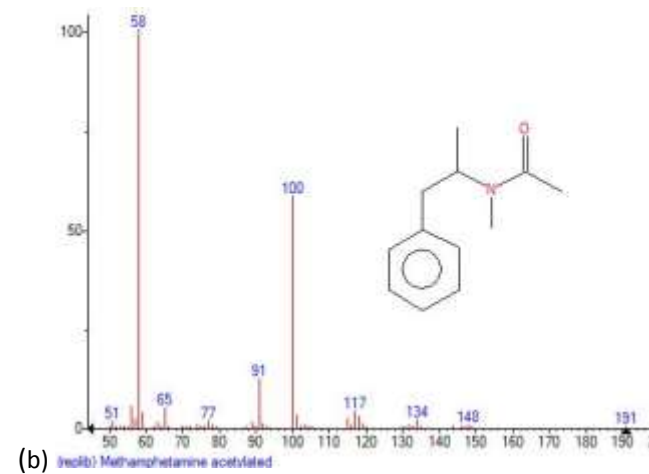
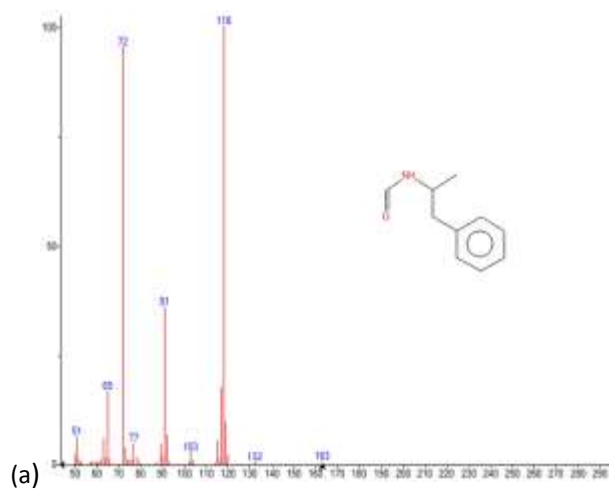


Figure 4.11 Chemical structures and mass spectra of four main pyrolysis products of methamphetamine (a) N-formylmethamphetamine (b) N-acetylmethamphetamine (c) dimethyl methamphetamine (d) methamphetamine

#### **4.5.3 Evaluation of the method repeatability for pyrolysis products of methamphetamine**

The repeatability of the method for the production of pyrolysis products of methamphetamine was evaluated to determine the analytical repeatability for desorbed samples. This was performed in two steps; the first was involved with the establishment of repeatability of results within one sampling tin (within-can repeatability) and the second was for the repeatability between the 6 six tin cans.

##### **4.5.3.1 General procedure for the evaluation of the method repeatability for pyrolysis products of nyaope street drug.**

Six replicate samples of the drug were prepared by weighing 0.5g of the powder material into six different tin cans and each sample pyrolysed. Each tin can contained 6 ACS strips. Overlays of chromatographic profiles of detected pyrolysis products were plotted and compared for retention time, peak shape, peak height as well as any differences in overall chemical profile. %RSD of peak areas for pyrolysis products from each tin can were calculated and used to represent analytical repeatability of products within each can (within-can repeatability). Analytical reproducibility was calculated using %RSD for average peak areas ( $n=6$ ) of pyrolysis products detected within each can compared across all 6 tin cans to generate the between can reproducibility ( $n=36$ ).

#### **4.5.4 Results and Discussions**

Typical chromatographic profiles of the detected pyrolysis products obtained from the 6 injections in tin can 1, (extracted using both MeOH-DCM 90:10 and MeOH-DCM 50:50 solvents at 354<sup>0</sup>C) are displayed in figures 4.12 and 4.13. They all show similar retention times, similar pyrolysis products and quantities as well as a large methamphetamine peak at 5.922 minutes. Table 4.3 and table 4.4 show calculated %RSDs for tin can 1 using MeOH-DCM 90:10 and MeOH-DCM 50:50 respectively.



Calculated %RSD for the analysis ranged between 0.8-5%, indicating excellent repeatability.

Chromatographic profiles of pyrolysis products generated across the six different tin cans were also compared. This was followed by the calculation of %RSDs across these samples. Figure 4.14 shows chromatographic profiles of methamphetamine pyrolysis products generated at different temperatures for the MeOH-DCM 50:50. Profiles revealed similar retention times but calculated %RSD values (table 4.5 and 4.6) were all  $\geq 5\%$ , indicating that repeatability was considerably more challenging. Plots of pyrolysis product versus product peak area (appendix 1; figures 4.15 and 4.16) for methamphetamine pyrolysis products also revealed variations in product quantities. This is not unreasonable given the nature of the pyrolysis event and that some variation would be expected to occur. The data is analogous to other between sample extraction data for synthetic drugs (for example extractions for different drug samples [177]).

Chromatographic profiles from both solvents extracts revealed a large methamphetamine at 5.22 minutes; a tentative explanation for the existence of the peak was that the methamphetamine might have undergone decomposition in the injection port of the GCMS where temperatures ( $280^{\circ}\text{C}$ ) were much higher than its melting point of  $170^{\circ}\text{C}$ .

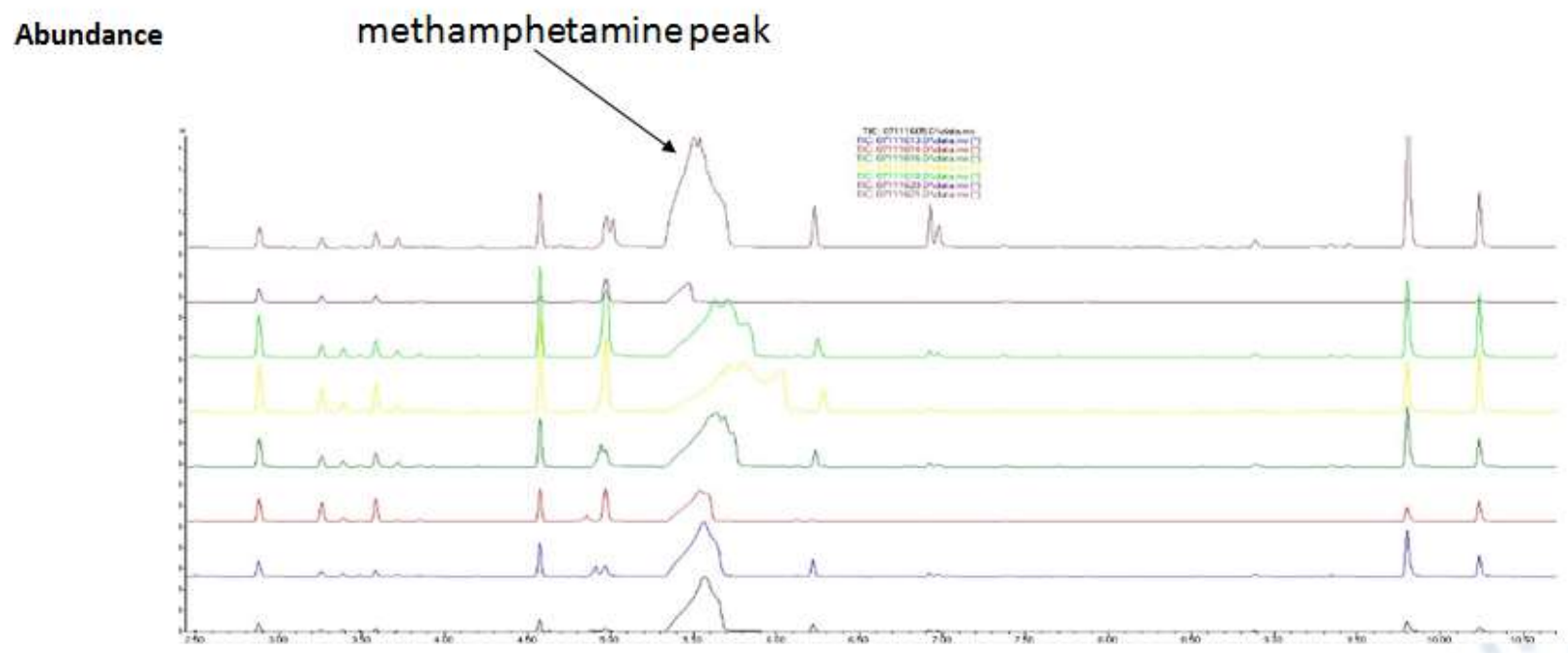


Figure 4.12 Overlay of chromatographic profile of pyrolysis products of methamphetamine extracted in MeOH-DCM 50:50 at 354°C, showing highlighted methamphetamine peak

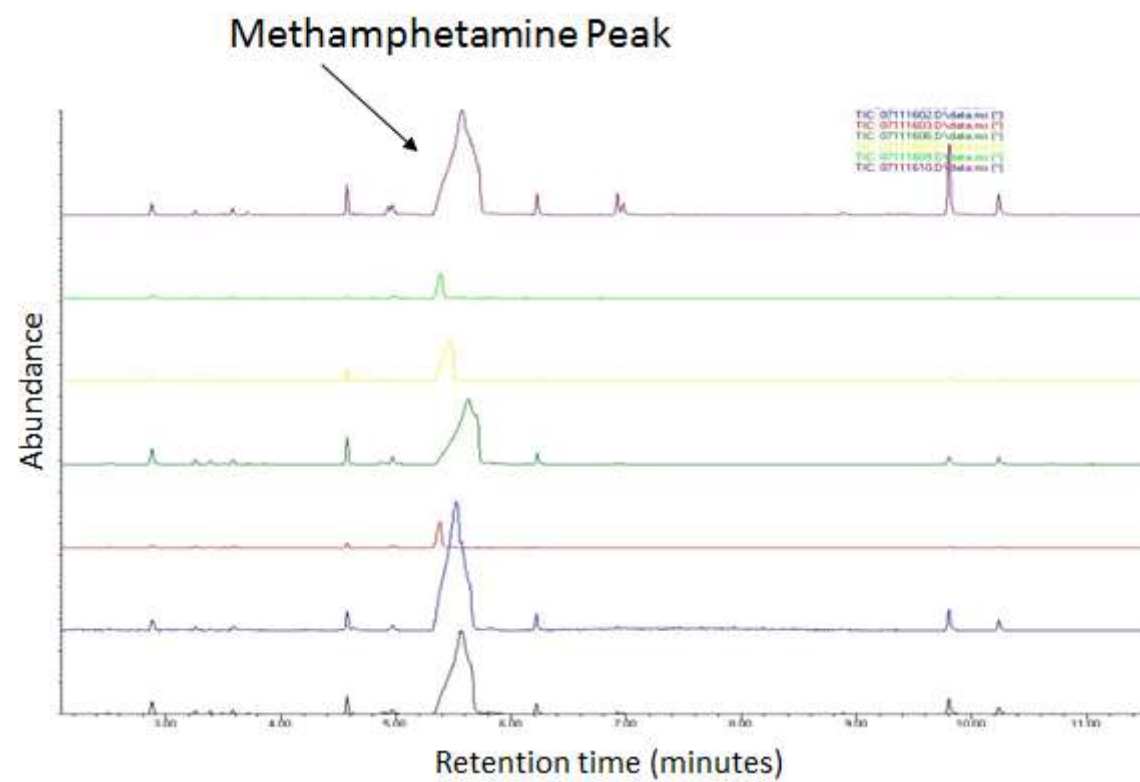


Figure 4.13 Overlay of chromatographic profile of pyrolysis products of methamphetamine extracted in MeOH-DCM 90:10 at 354<sup>0</sup>C, also showing the methamphetamine peak

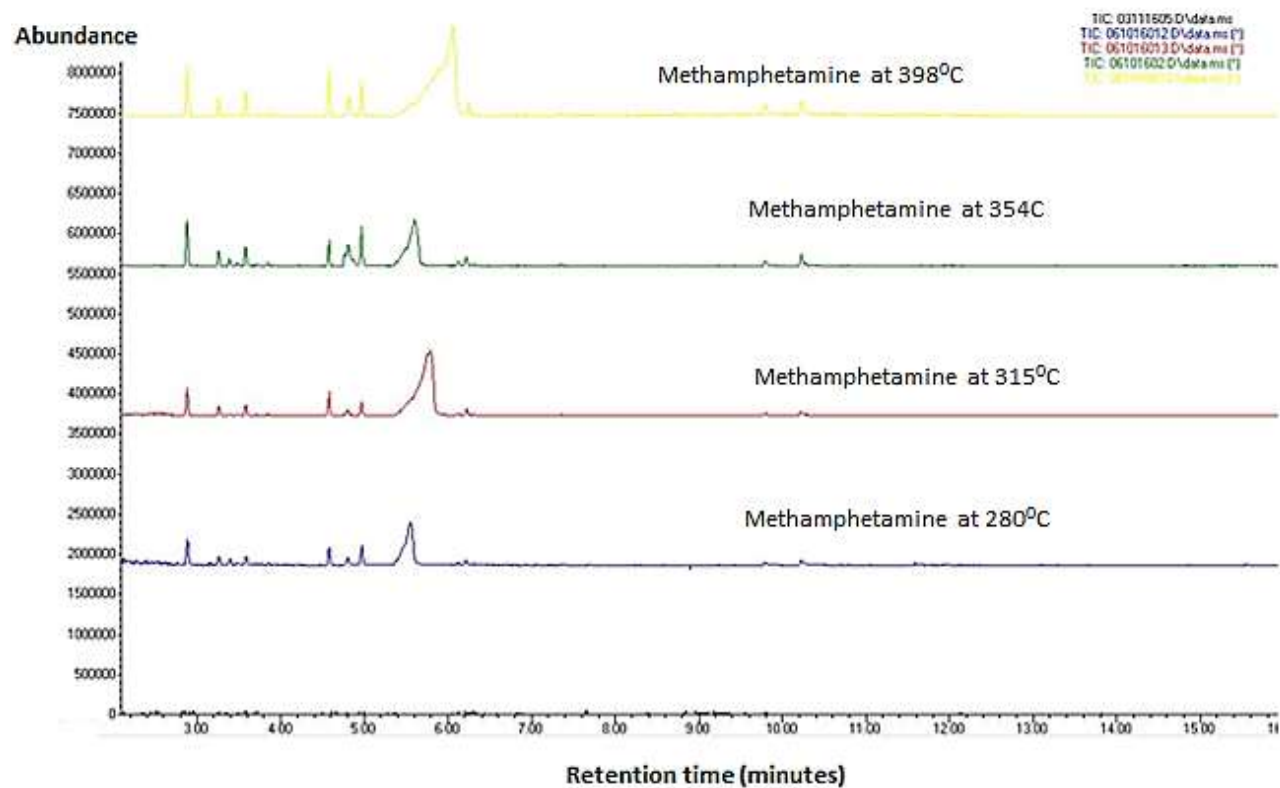


Figure 4.14 Overlay of chromatographic profile of pyrolysis products of methamphetamine extracted in MeOH-DCM 50:50 at different temperatures

**Table 4.3 %RSD results for pyrolysis products of methamphetamine for within-can analytical repeatability extracted in MeOH-DCM 50:50 at 354<sup>0</sup>C**

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in each ACS in tin can 1								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	stdev	%RSD
Benzaldehyde	5935732	5905670	5808374	5945662	5976632	5953273	<b>5920891</b>	<b>59801.78</b>	<b>1.0</b>
Cyclo propyl benzene	187202	187667	179201	192113	186632	186421	<b>186539</b>	<b>4162.94</b>	<b>2.2</b>
Benzyl methyl ketone	2438910	2454461	2499620	2400371	2679335	2388356	<b>2476842</b>	<b>106901</b>	<b>4.3</b>
Phenyl-1,2 propanedione	6015307	6002503	5937219	6015307	6321003	6221007	<b>6085391</b>	<b>150035.7</b>	<b>2.4</b>
N,alpha-dimethyl benzeneethanamine	142421300	143257324	152100037	156222871	142654922	139611784	<b>14600000</b>	<b>6542687</b>	<b>4.4</b>
Benzoin	6015307	6021151	6103208	6000366	6116201	6014287	<b>6045087</b>	<b>50682.62</b>	<b>0.8</b>
N-formylmethamphetamine	19274238	23797551	20357559	18999534	19573560	22887621	<b>21148344</b>	<b>1086695</b>	<b>5.4</b>
N-acetylmethamphetamine	8595102	9126062	8699452	9003245	7981503	7960622	<b>8727664</b>	<b>425634.1</b>	<b>4.9</b>
Trans 1,2- phenyl-dimethyl Aziridine	142421300	140539771	138750026	141538382	127755024	142260025	<b>139000000</b>	<b>5613718</b>	<b>4.0</b>

**Table 4.4 %RSD results for pyrolysis products of methamphetamine for within-can analytical repeatability extracted in MeOH-DCM 90:10 at 3540C**

Pyrolysis product	Pyrolysis product average peak area from the six replicate injections in each ACS in tin can 1								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
Benzaldehyde	1050792	1050795	1049115	1050563	1056665	1066672	<b>1054100</b>	<b>6691</b>	<b>1</b>
Cyclo propyl benzene	65821	64823	65779	68255	65773	66653	<b>66184</b>	<b>1168</b>	<b>2</b>
1 phenyl 1,2 propanedione	94159	94237	94144	96511	93674	95632	<b>94726</b>	<b>1097</b>	<b>1</b>
Benzeneethanamine, N, alpha-dimethyl	3356342	3276001	3354212	3610110	3346778	3330089	<b>3378922</b>	<b>117114</b>	<b>3</b>
Benzoin	362394	364577	357782	362001	359912	365446	<b>362019</b>	<b>2857</b>	<b>1</b>
N-formylmethamphetamine	81975	81972	79834	81432	79229	82411	<b>81142</b>	<b>1299</b>	<b>2</b>
N-acetylmethamphetamine	15502	14502	16221	14324	15443	14456	<b>15075</b>	<b>762</b>	<b>5</b>

**Table 4.5 %RSD results for pyrolysis products of methamphetamine for inter-can analytical repeatability extracted in MeOH-DCM 50:50 at 354<sup>0</sup>C**

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in each ACS in tin can 1								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
Benzaldehyde	5935732	1365941	10206219	764771	15636920	5591527	<b>6583518</b>	<b>5613780</b>	<b>85</b>
Cyclo propyl benzene	187202	290689	295616	515748	882807	247752	<b>403302.33</b>	<b>259900.80</b>	<b>64</b>
Benzyl methyl ketone	2438910	2042030	25448383	14552407	76495875	5631814	<b>21101569.83</b>	<b>28569877.98</b>	<b>135</b>
Phenyl-1,2 propanedione	6015307	1149236	11607074	22464220	17791319	3523059	<b>10425035.83</b>	<b>8411001.75</b>	<b>81</b>
N,alpha-dimethyl benzeneethanamine	142421300	22135588	33848492	36081781	25701954	25571440	<b>47626759.17</b>	<b>46746264.30</b>	<b>98</b>
Benzoin	6015307	370282	5407958	7196611	1137748	2674340	<b>3800374.33</b>	<b>2798132.99</b>	<b>74</b>
N-formylmethamphetamine	19274238	287069	815823	838411	41867032	4131266	<b>11202306.5</b>	<b>16672809.37</b>	<b>149</b>
N-acetylmethamphetamine	8595102	149025	80713	377955	6130425	916748	<b>2708328</b>	<b>3700260.68</b>	<b>137</b>
Trans 1,2- phenyl-dimethyl Aziridine	142421300	6629139	12204525	18005572	19868903	11719515	<b>35141492.33</b>	<b>52770250.46</b>	<b>150</b>

**Table 4.6 %RSD results for pyrolysis products of methamphetamine of inter-can analytical repeatability extracted in MeOH-DCM 90:10 at 354<sup>0</sup>C**

Pyrolysis product	Pyrolysis product peak area								
	can1	can2	can3	can 4	can 5	can 6	Average	Stdev	%RSD
Benzaldehyde	1050792	605269	ND	1564535	991098	ND	<b>1052924</b>	<b>394110</b>	<b>37</b>
Cyclo propyl benzene	65821	ND	ND	138001	ND	ND	<b>101911</b>	<b>51039</b>	<b>50</b>
1 phenyl 1,2 propanedione	94159	178230	23506	266167	ND	30880	<b>118588.4</b>	<b>103280</b>	<b>87</b>
Benzeneethanamine, N, alpha-dimethyl	3356342	5137359	1778845	759036	497962	457002	<b>1997758</b>	<b>1892539</b>	<b>95</b>
Benzoin	362394	257226	69615	513619	548879	55895	<b>301271.3</b>	<b>212580</b>	<b>71</b>
N-formylmethamphetamine	81975	49855	14474	104293	115067	22656	<b>64720</b>	<b>42222</b>	<b>62</b>
N-acetylmethamphetamine	15502	11348	2299	17148	18313	ND	<b>12922</b>	<b>6498</b>	<b>50</b>



#### 4.6 Determination of methamphetamine synthetic routes from detected pyrolysis products

The detection of methamphetamine amongst its pyrolysis products indicates the possibility of incomplete hydrolysis or a breakdown product from the intermediate product N-Formylmethamphetamine[166]. Other products, Dimethyl amphetamine, Acetyl-methamphetamine, amphetamine, N-methylamphetamine and N-formylmethamphetamine are main pyrolysis products of methamphetamine produced via reactions of N-formylation, N-acetylation and N-demethylation. These have been previously reported [178-186].

The detection of benzaldehyde and ally benzene in all samples is indicative of their use in the synthesis of 1-phenyl-2-propanone (P2P), a starting material for the production of methamphetamine via the Leuckart route [5] (figure 4.17-18). As an internationally monitored chemical, P2P has become increasingly difficult for illicit amphetamine laboratories to secure, which means alternative routes for its manufacture have become increasingly important. New synthetic route impurities are therefore highly likely. A variety of P2P manufacturing chemicals in methamphetamine samples have been reported [6].

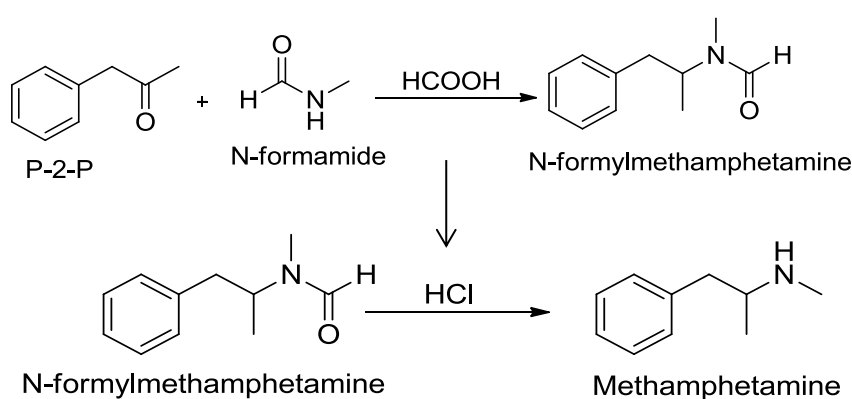
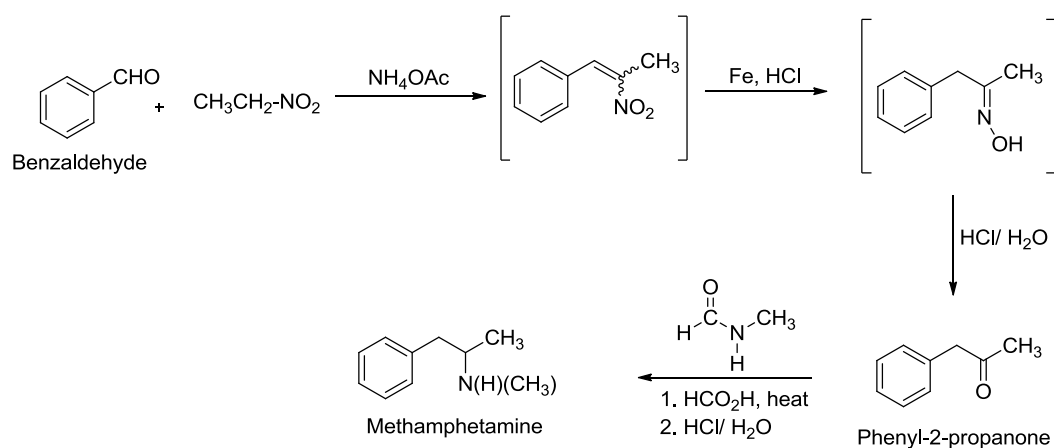


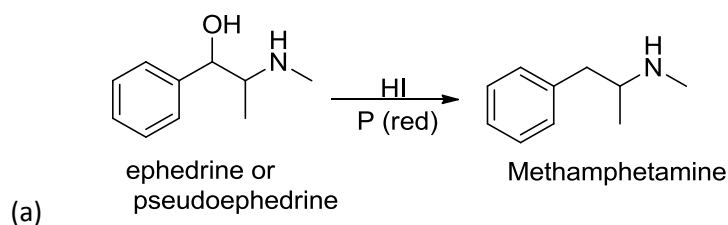
Figure 4.17 Methamphetamine synthesis through the Leuckart route [169]

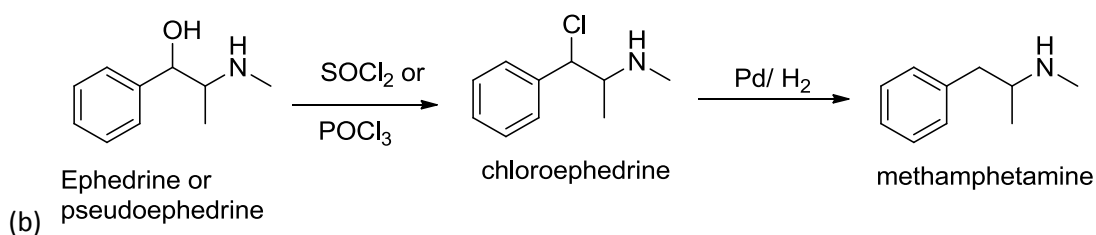


**Figure 4.18 P2P synthesis using benzaldehyde [170]**

The reaction between P2P and N-methylformamide produces an intermediate, N-formylmethamphetamine [170], which was detected in all samples extracted with Methanol-dichloromethane 50:50 v/v. Allyl benzene (propenyl benzene) can be used as a precursor for the Leuckart methamphetamine synthesis through its treatment hydrogen bromide. The reaction produces an intermediate, 1-phenyl-2-bromopropane, which can be converted to methamphetamine [167,171]. Even though this intermediate was not detected, allyl benzene was detected in all samples, suggesting that it was used as a starting material for P2P.

The presence of the methamphetamine isomer suggests the use of ephedrine or pseudoephedrine as starting materials via the Emde or Nagai routes [172-174] (figure 4.19a and b).





**Figure 4.19 Methamphetamine synthesis via the two routes of (a) Nagai and (b) Emde**

Both (+)-ephedrine and (+)-norephedrine were also detected. The two synthesis methods are both associated with the production of aziridine type impurities [170, 173-175]; trans 1,2 dimethyl-3-phenylaziridine was detected in all samples. Since these impurities are common to both methods, their presence cannot be considered as being route specific, unless they accompanied by other products which are thought to be more specific, for example, dimethyl naphthalene for the Nagai route [170, 171]. Only the ephedrine and aziridine type impurities were detected in this study. Studies involving the determination of pyrolysis products of methamphetamine have been performed in the past; amongst these were some which revealed the presence of chemicals from precursor materials, intermediate or by-products of chemical reactions used during the synthesis. The types of products produced were largely dependent on the synthetic route employed [171-179]. Some studies even went further to use the detected pyrolysis products to discriminate between synthetic routes with common starting materials [174, 180]. Based on the results obtained, it was concluded that there is a possibility that the methamphetamine samples used for this research were synthesised via the Leuckart, Emde or Nagai synthetic routes. Similar pyrolysis products have also been reported from methamphetamine samples synthesised via the three synthetic routes [165, 181]

A side by side comparison of commonly encountered pyrolysis products of methamphetamine (table 4.7) was performed against detected pyrolysis products (table 4.8) to confirm possible synthetic routes for samples used in this research.

**Table 4.7 Commonly encountered synthetic impurities for three methamphetamine synthetic routes [177]**

<b>Emde synthetic route</b>	<b>Nagai synthetic route</b>	<b>Leuckart route</b>
Benzaldehyde Cis/trans-1,2 –dimethyl -3-phenylaziridine Dimethylamphetamine Ephedrine Chloroephedrine N-formylamphetamine N-acetylmethamphetamine Benzylmethamphetamine N-benzoylmethamphetamine Methylamphetamine dimer 3,4 Dimethyl-5-phenyloxazolidine	Amphetamine Dimethylamphetamine N-formylmethamphetamine N-formylamphetamine N-acetylamphetamine Benzylmethamphetamine N-benzoylamphetamine Benzylmethnaphthalene Bibezyll Cis/trans-1,2 –dimethyl -3-phenylaziridine N-benzoylmethamphetamine Trans/cis-3,5-Dipheny-3-buten-one Dimethylphenylaphthalenel Ephedrine Chloroephedrine	Amphetamine Dimethylamphetamine N-formylamphetamine Dimethylamphetamine Bibezyll N-acetylmethamphetamine N-formylamphetamine Dibenzylketone Benzylmeththylamphetamine trans 3.4-Diphenyl-3-buten-2-one N-benzoylamphetamine Benzaldehyde 1-phenyl-2-propanone

**Table 4.8 Detected pyrolysis products of methamphetamine matched with three possible synthetic routes**

ACS extracting solvent	Pyrolysis Temperature (°C)		
	315	354	396
MeOH:DCM 50:50	N-Formylmethamphetamine N-Methylbenzylamide trans 1,2 Dimethyl -3-phenylaziridine Allylbenzene Benzaldehyde	N-Formylmethamphetamine N-Methylbenzylamide norephedrine Deoxyephedrine 1-Phenyl-1-Propane Trans 1,2-dimethyl-3-phenyl Aziridine 1-propenyl benzene Benzaldehyde	N-Formylmethamphetamine norephedrine Methoxyphenamine 1-Phenyl-2-Propanone trans 1,2-dimethyl-3-phenyl Aziridine 1-propenylbenzene Cyclopropylbenzene Benzaldehyde
MeOH:DCM 90:10	N-Methylbenzylamide trans 1,2 Dimethyl -3-phenylaziridine Methamphetamine Amphetamine Cyclopropylbenzene Benzoic acid Allylbenzene Benzaldehyde Ephedrine N-acetylmethamphetamine Dimethylamphetamine N-methylamphetamine	Methamphetamine 1-Phenyl-2-Propanone 1-Propenylbenzene Benzaldehyde cyclopropylbenzene N-acetylmethamphetamine Dimethylamphetamine	Ephedrine Deoxyephedrine Ethyl phenyl ketone Trans 1,2-dimethyl-3-phenyl Aziridine 1-Propenylbenzene Benzaldehyde

Colour key: yellow = Leuckart route, green = common to Emde and Nagai routes, blue = common to all three synthetic routes

#### 4.7 Isolation of pyrolysis products of EFV and methamphetamine, and analysis with the GCMS.

Six replicate samples of EFV and methamphetamine were prepared by weighing 0.5g of each drug and mixing the two drugs into 6 separate tin cans. The mixture was heated to 354<sup>OC</sup> and pyrolysis products extracted according to the protocol described chapters 4.2.2.1. The products were trapped using four ACS in each of the six tin cans. The analysis sampling strategy in Chapter 4.2.2.2 was applied where one of the four ACS strips was extracted with MeOH-DCM 90:10 and the other with MeOH-DCM 50:50. Methamphetamine samples were in-house samples synthesised for research purposes.

#### 4.7.1 Results and Discussions

The results obtained (table 4.9) revealed the presence of benzaldehyde, cyclopropylbenzene, 1-phenyl-1,2-propanedione, N, alpha-dimethyl benzeneethanamine (methamphetamine), Benzoin, N-formylmethamphetamine, N-acetyl methamphetamine, EFV pyrolysis product 1 and EFV. Typical chromatographic profiles of pyrolysis products obtained from the pyrolysis of the mixture with both solvent ratios are displayed in figures 4.20 and 4.21. Detected pyrolysis products have been reported as impurities arising from various chemicals and intermediate products of reactions used during the synthesis of methamphetamine. Similar pyrolysis products have been reported [167, 168]

**Table 4.9 Pyrolysis products of EFV and methamphetamine extracted in the two solvents of MeOH-DCM**

ACS extracting solvent	Pyrolysis Temperature (°C)	
	315	354
<b>MeOH:DCM 50:50</b>	Methamphetamine N-Formylamphetamine N-Acetylamphetamine 2-furan methanol	Benzaldehyde Cylopropylbenzene 1-phenyl-1,2,-propanedione N-alpha-dimethyl- benzeneethanamine Benzoin N-formylmethamphetamine N-acetylmethamphetamine EFV EFV pyrolysis product 1
<b>MeOH:DCM 90:10</b>	methamphetamine 1,2-Propanediol, 1-phenyl Benzaldehyde N- Acetylamphetamine N-Formylamphetamine 2-furan methanol	Benzaldehyde Cylopropylbenzene 1-phenyl-1,2,-propanedione N-alpha-dimethyl- benzeneethanamine Benzoin N-formylmethamphetamine N-acetylmethamphetamine EFV EFV pyrolysis product 1



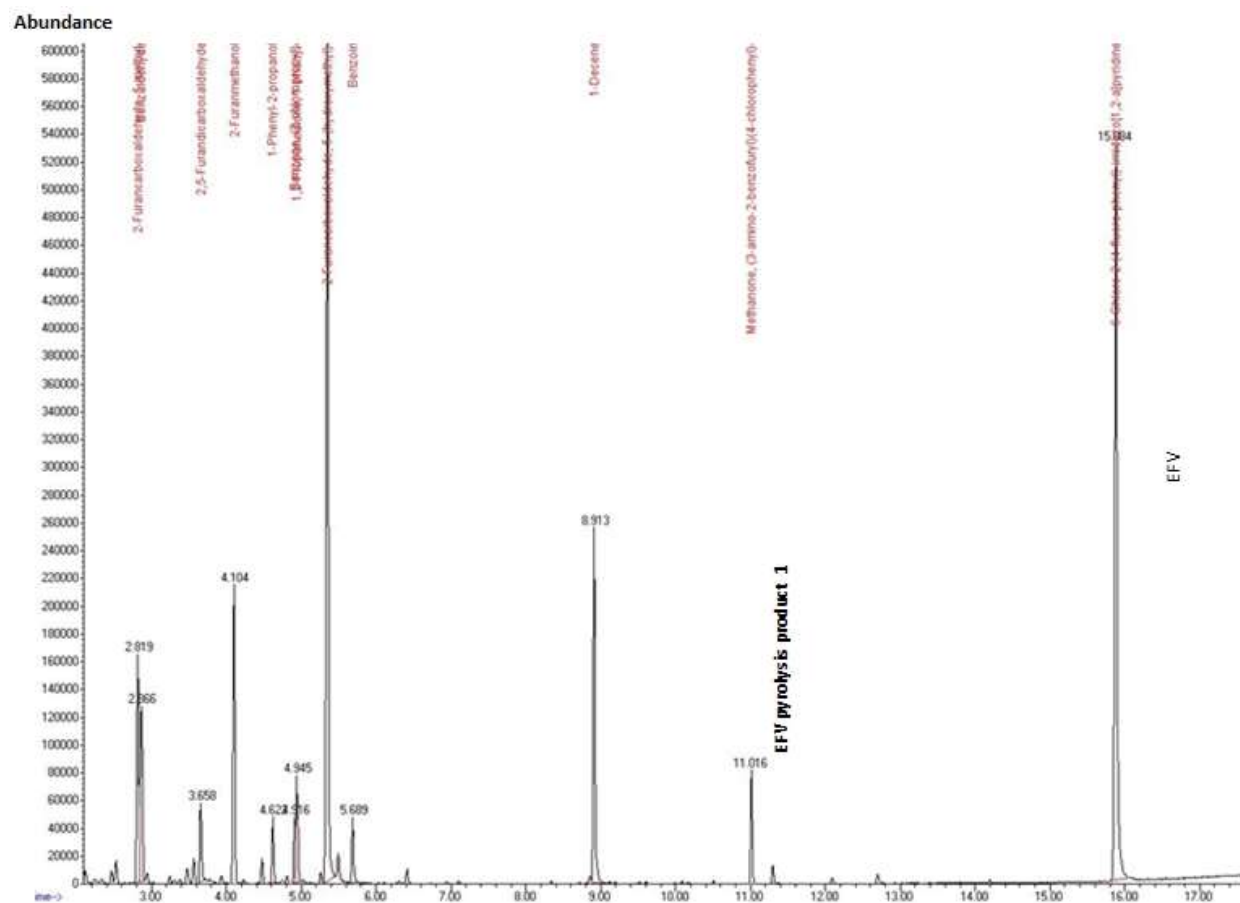


Figure 4.21 Chromatogram for pyrolysis products of EFV-methamphetamine extracted in MeOH-DCM at 354<sup>0</sup>C



#### **4.7.2 Evaluation of method repeatability for EFV and methamphetamine**

The repeatability of the method for the production of pyrolysis products of EFV and methamphetamine was evaluated to determine the analytical repeatability for desorbed samples. This involved the establishment of analytical repeatability of results within one sampling tin (within-can repeatability) and between 6 six tin cans (inter-can repeatability). For within-can repeatability, two sets of 6 replicate samples were prepared as described previously in section 4.5.6, desorbing the ACS using the appropriate solvent. Each resultant extract was analysed six times to facilitate the calculation of repeatability within a desorption and analysis event. Chromatographic profiles of pyrolysis products generated from each of the six replicate injections in each sample can were plotted and compared for similarities in retention time, peak shape, peak height as well as any differences in overall chemical profile.

Within-can repeatability was evaluated through %RSDs values calculated from average peak areas of pyrolysis products detected from the 6 replicate injections performed on each of the 6 strips in each tin can using both solvents and ratios.

%RSD results for within-can repeatability for each pyrolysis product obtained from the 6 cans were compiled (average peak areas of pyrolysis products detected from the 6 injections in each of the 6 tin cans (n=36); their %RSDs values calculated and used to establish repeatability.

##### **4.7.2.1 Repeatability results**

Typical chromatographic profiles of the detected pyrolysis products obtained from the 6 injections in tin can 1, (extracted using both MeOH-DCM 90:10 and MeOH-DCM 50:50 solvents at 354<sup>0</sup>C) are displayed in figures 4.22 and 4.23. They all show similar retention times, similar pyrolysis products and quantities. Table 4.10 and table 4.11 show calculated %RSDs for tin can 1 using MeOH-DCM 90:10 and MeOH-DCM 50:50 respectively. Calculated %RSDs for the analysis was all  $\leq 5\%$ , indicating excellent repeatability.

Chromatographic profiles of pyrolysis products generated across the six different tin cans were also compared. This was followed by the calculation of %RSDs across these samples. Figure 4.24 and 4.25 shows chromatographic profiles of methamphetamine pyrolysis products generated at 354<sup>0</sup>C for the MeOH-DCM 50:50 and 90:10 solvents respectively. Profiles revealed similar retention times but calculated %RSD values (table 4.12 and 4.13) were all  $\geq 5\%$ , indicating that repeatability was not achievable. Plots of pyrolysis product versus product peak area (figures 4.25 and 4.26) for EFV-methamphetamine pyrolysis products also revealed variations in product quantities. This was expected given the nature of the pyrolysis event and that some variation was probable.

Chromatographic profiles from both solvents extracts revealed a large methamphetamine peak at 5.22 minutes; a tentative explanation for the existence of the peak was that the methamphetamine might have undergone decomposition in the injection port of the GCMS where temperatures (280<sup>0</sup>C) were much higher than its melting point of 170<sup>0</sup>C.

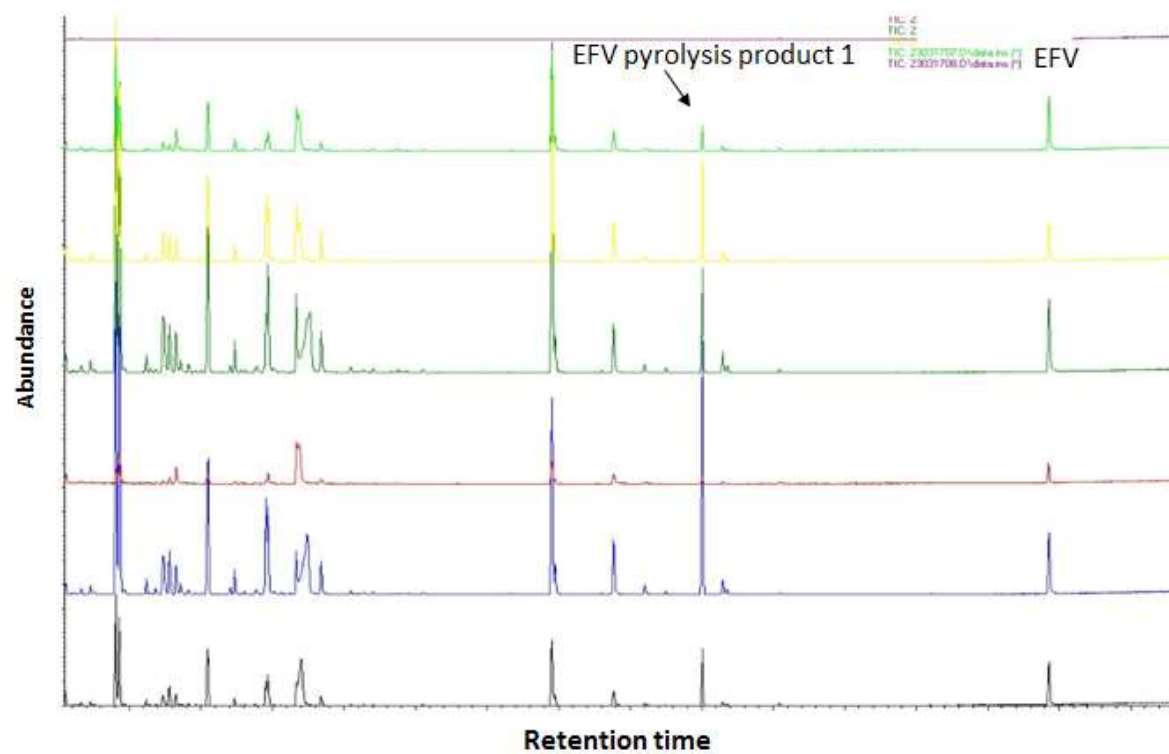


Figure 4.22 Overlay of chromatographic profile of pyrolysis products of EFV-methamphetamine extracted in MeOH-DCM 50:50 at 354<sup>0</sup>C, showing EFV pyrolysis product 1 at 11 minutes and EFV at 16 minutes

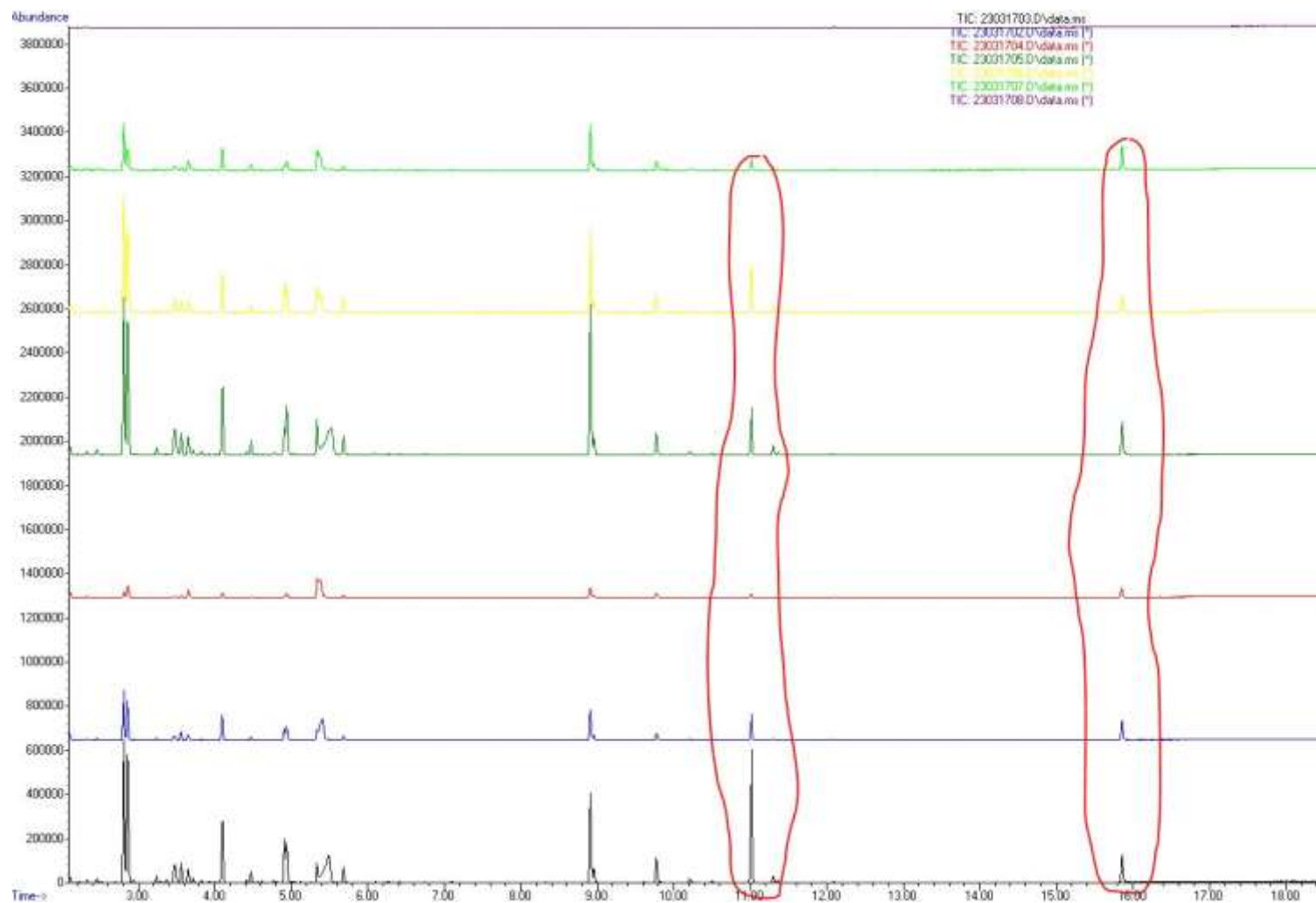


Figure 4.23 Overlay of chromatographic profile of pyrolysis products of EFV-methamphetamine extracted in MeOH-DCM 90:10 at 354<sup>0</sup>C, showing EFV pyrolysis product 1 at 11 minutes and EFV at 16 minutes

**Table 4.10 %RSD results for pyrolysis products of EFV a and methamphetamine for within-can analytical repeatability extracted in MeOH-DCM at 354<sup>0</sup>C**

Pyrolysis product	Average abundance								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	RSD%
2-Furan carboxaldehyde-5 methyl	2851396	2846775	2956734	2855942	2854937	2858657	<b>2870740</b>	<b>42327.75</b>	<b>1.47</b>
Benzaldehyde	2573923	2550275	2497332	2518932	2815465	2836651	<b>2632096</b>	<b>152652</b>	<b>5.80</b>
Cyclo propyl benzene	486615	489769	491022	149673	157108	485698	<b>4894918</b>	<b>4079.33</b>	<b>0.83</b>
2-furan methanol	2900269	2981700	2914634	2932105	2944837	2897553	<b>2928516</b>	<b>31802.84</b>	<b>1.09</b>
1,2-propanedione ,1-phenyl	1571980	1577231	1518731	1581627	1541354	1551380	<b>1557051</b>	<b>24417.48</b>	<b>1.57</b>
Benzeneethanamine, N, alpha-dimethyl	3825507	3762834	3857710	3951127	3960392	3944002	<b>3883595</b>	<b>80913.32</b>	<b>2.08</b>
Benzoin	284996	294226	283353	296007	294326	297644	<b>291759</b>	<b>6029.15</b>	<b>2.07</b>
1-Dodecanol	1489188	1474117	1477756	1567110	1403954	1572892	<b>1497503</b>	<b>63696.22</b>	<b>4.25</b>
N-Formylmethamphitane	1493846	1554567	1576433	1540475	1622712	1645290	<b>1572221</b>	<b>55440.55</b>	<b>3.53</b>
methamphetamine acetylated	60946	65731	69302	67533	68619	69445	<b>66929</b>	<b>3237.79</b>	<b>4.84</b>
EFV product 1	1571366	1558265	1473425	1576835	1567562	1496767	<b>1540703</b>	<b>44118.64</b>	<b>2.86</b>
Trichloroctic acid pentadecyl ester	26239	27301	25787	27722	28736	26986	<b>27129</b>	<b>1056.14</b>	<b>3.89</b>
EFV	1191516	1176214	1136774	1267129	1139856	1125640	<b>1172855</b>	<b>52663.33</b>	<b>4.49</b>

**Table 4.11 %RSD results for EFV-methamphetamine pyrolysis product vs peak area for within-can analytical repeatability extracted in MeOH-DCM 90:10 at 354<sup>o</sup>C**

Pyrolysis product	Average peak area								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	average	Stdev	%RDS
Benzaldehyde	1050792	1050792	1050700	1050699	1052792	1050789	<b>1051094</b>	<b>833.05</b>	<b>0.08</b>
cyclopropylbenzene	65821	65707	65722	65800	65820	65819	<b>65781.5</b>	<b>52.691</b>	<b>0.08</b>
Benzyl methyl ketone	145351	145366	145356	145344	145319	145366	<b>145350.3</b>	<b>17.580</b>	<b>0.01</b>
1-phenyl-1,2 propanedione	94159	94150	94146	94146	94148	94152	<b>94150.17</b>	<b>4.92</b>	<b>0.01</b>
N, alpha-dimethyl Benzeneethanamine	3356342	3356771	3356317	3356364	3361223	3366212	<b>3358872</b>	<b>4075.19</b>	<b>0.12</b>
N-formylmethamphetamine	362394	362393	362394	363212	362336	355895	<b>361437.3</b>	<b>2735.63</b>	<b>0.76</b>
N-acetylmethamphetamine	81975	81973	81935	81987	81974	81988	<b>81972</b>	<b>19.31</b>	<b>0.02</b>
EFV	15502	15503	15521	15491	15511	15507	<b>15505.83</b>	<b>10.01</b>	<b>0.06</b>
EFV pyrolysis product 1	89254	89253	89255	89253	89249	89253	<b>89252.83</b>	<b>2.04</b>	<b>0.002</b>

**Table 4.12 %RSD results for EFV- methamphetamine pyrolysis products vs peak area for inter-can analytical repeatability extracted in MeOH-DCM at 354°C**

Pyrolysis product	Average abundance								
	can 1	can 2	can 3	can 4	can 5	can 6	Average	Stdev	RSD%
2-Furan carboxaldehyde-5 methyl	2851396	9046785	322984	10486912	7754939	2858657	<b>5553612</b>	<b>4081906</b>	<b>73.5</b>
Benzaldehyde	2573923	8450286	731102	9331353	5815533	2.863	<b>4483670</b>	<b>3970897</b>	<b>88.6</b>
Cyclo propyl benzene	486615	769299	153622	1230140	767158	174098	<b>596822</b>	<b>411612</b>	<b>68.9</b>
2-furan methanol	1500269	3981700	294634	4330285	2444837	1475213	<b>2337823</b>	<b>1568688</b>	<b>67.1</b>
1,2-propanedione ,1-phenyl	1571980	2811710	201873	3610286	3254230	930380	<b>2161696</b>	<b>1505955</b>	<b>69.67</b>
Benzeneethanamine, N, alpha-dimethyl	3825507	628129	3554710	2119527	3960392	3221012	<b>2696754</b>	<b>1343514</b>	<b>49.82</b>
Benzoin	284996	907596	132253	1161703	942164	236103	<b>675964</b>	<b>460857</b>	<b>68.18</b>
1-Dodecanol	1489188	4746496	50308	8110480	4618354	2502892	<b>4005706</b>	<b>2986507</b>	<b>74.56</b>
N-Formylmethamphitane	1493846	1615700	1258572	1504758	1302490	1645290	<b>1465362</b>	<b>177353</b>	<b>12.10</b>
methamphetamine acetylated	60946	274051	33220	238822	189619	55074	<b>158157</b>	<b>108586</b>	<b>68.66</b>
EFV product 1	1571366	8558683	207415	3019014	2892068	702031	<b>3075842</b>	<b>3315170</b>	<b>107.78</b>
Trichlorocetic acid pentadecyl ester	26239	109062	2470	129922	71673	26986	<b>680236</b>	<b>53643</b>	<b>78.86</b>
EFV	1191516	2124485	539686	2448129	1061881	1685140	<b>1571864</b>	<b>776456</b>	<b>49.40</b>

**Table 4.13 %RSD results for EFV-methamphetamine vs peak area for inter-can analytical repeatability extracted in MeOH-DCM at 354<sup>0</sup>C**

Pyrolysis product	Average peak area								
	Can 1	Can 2	Can 3	Can 4	Can5	Can 6	average	Stdev	%RDS
Benzaldehyde	1050792	605269		1564535	991098	-	<b>1052923.5</b>	<b>394109.89</b>	<b>37.43</b>
cyclopropylbenzene	65821	-	-	138001	-	-	<b>101911</b>	<b>51038.97</b>	<b>50.08</b>
Benzyl methyl ketone	145351	79167	-	224942	-	38659	-	-	-
1-phenyl-1,2 propanedione	94159	178230	23506	266167		30880	<b>118588.4</b>	<b>103279.96</b>	<b>87.09</b>
N, alpha-dimethyl Benzeneethanamine	3356342	5137359	1778845	759036	497962	457002	<b>1997757.67</b>	<b>1892538.88</b>	<b>94.73</b>
N-formylmethamphetamine	362394	257226	69615	513619	548879	55895	<b>301271.33</b>	<b>212579.99</b>	<b>70.56</b>
N-acetylmethamphetamine	81975	49855	14474	104293	115067	22656	<b>64720</b>	<b>42222.24</b>	<b>65.24</b>
EFV	15502	11348	2299	17148	18313	-	<b>12922</b>	<b>6498.15</b>	<b>50.29</b>
EFV pyrolysis product 1	89254	120374	3672	121302	75984	18024	<b>71435</b>	<b>50327.95</b>	<b>70.45</b>



#### **4.8 GCMS conclusions about the pyrolysis of EFV and methamphetamine**

The results obtained from the analysis of pyrolysis products of methamphetamine and from a mixture of the two drugs revealed the presence of products from various chemicals used as starting materials and in the making of other chemicals for the manufacture of methamphetamine. The detected pyrolysis products in this case were used to establish possible synthetic routes for the samples used. It is thought the detected products only survived the pyrolysis process because they were in excess quantities or due to incomplete chemical reactions (where they were detected as intermediate products). Product quality is usually compromised in clandestine laboratory settings because most chemicals used are controlled. It was also discovered that analytical repeatability of desorbed pyrolysis products was only achievable for products produced within one tin can and not between two or more different tin cans. This is as a result of the unpredictability of the generation of pyrolysis products and was to be expected.

The presence of EFV in the mixture did not interfere with the pyrolysis patterns of methamphetamine and vice versa. The overall conclusion relating to the smoking of the two drugs together as mixture is that the pyrolysis products will contain a mixture of products from both drugs.

#### **4.9 Isolation of pyrolysis products of methamphetamine and analysis with the HPLC**

##### **4.9.1 Sample preparation**

Methamphetamine samples used for the HPLC analysis were the two remaining ACS strips from the four prepared earlier in section 4.3 where six replicate methamphetamine samples were homogenised and prepared following the protocol described in chapters 4.2.2.1; 0.5g of the powdered material was weighed into 6 separate tin cans, heated and pyrolysis products extracted. The products were trapped using four ACS in each of the six tin cans. Following this, the analysis sampling strategy in Chapter 4.2.2.2 was applied where two of the strips were used for GCMS analysis and two by the HPLC. One of the two strips was extracted with

the MeOH-DCM 90:10 while the other with the mobile phase. Methamphetamine samples were in-house samples synthesised for research purposes.

#### **4.9.2 Results and discussions**

Results obtained from extractions using the mobile phase are displayed in table 4.14 and figure 4.26 while figure 4.27 and Table 4.15 shows results for the MeOH-DCM 90:10. Both results revealed the detection of the same pyrolysis products being a mixture of benzaldehyde, propenyl benzene, P2P, methamphetamine, dimethyl amphetamine, N-formylmethamphetamine, N-acetylmethamphetamine and trans-1, 2-dimethyl-3-phenylaziridine. All the detected pyrolysis products have been discussed in the previous section where they were discovered to be chemicals used as starting materials in the different synthetic routes mentioned earlier or intermediate products from chemical reactions involved.

**Table 4.14 Pyrolysis products of methamphetamine detected following extraction the mobile phase at 354°C**

Pyrolysis Temperature ( in degrees Celsius)	HPLC methamphetamine	
	Mobile phase 25Mm buffer-ACN (53:47)	Retention time (minutes)
315	Benzaldehyde Allylbenzene 1,2 Dimethyl -3-phenylaziridine,trans P2P Methamphetamine di-methyl amphetamine N-formyl methamphetamine N-acetylmethamphetamine	1.261 1.641 2.349 2.458 3.256 3.810 11.468 11.748
354	Benzaldehyde Allylbenzene 1,2 Dimethyl -3-phenylaziridine,trans P2P Methamphetamine di-methyl amphetamine N-formyl methamphetamine N-acetylmethamphetamine	1.261 1.641 2.349 2.458 3.256 5.483 11.468 11.748
358	Benzaldehyde Allylbenzene 1,2 Dimethyl -3-phenylaziridine,trans P2P Methamphetamine di-methyl amphetamine N-formyl methamphetamine N-acetylmethamphetamine	1.261 1.641 2.349 2.458 3.256 5.483 11.468 11.748

**Table 4.15 Results for pyrolysis products of methamphetamine extracted in MeOH-DCM 90:10 at 354<sup>0</sup>C**

Pyrolysis Temperature (in degrees Celsius)	HPLC methamphetamine	
	MeOH-DCM 9010 v/v	Retention time (minutes)
315	Allylbenzene	1.67
	Trans 1,2 dimethyl -3-phenylaziridine	2.40
	P2P	2.52
	Methamphetamine	3.38
	di-methyl amphetamine	3.97
	N-formyl methamphetamine	12.29
	N-acetylmethamphetamine	12.61
354	Allylbenzene	1.67
	Trans 1,2 Dimethyl -3-phenylaziridine	2.40
	P2P	2.52
	Methamphetamine	3.38
	di-methyl amphetamine	3.97
	N-formyl methamphetamine	12.29
	N-acetylmethamphetamine	12.61
358	Allylbenzene	1.67
	Trans 1,2 Dimethyl -3-phenylaziridine	2.40
	P2P	2.52
	Methamphetamine	3.38
	di-methyl amphetamine	3.97
	N-formyl methamphetamine	12.29
	N-acetylmethamphetamine	12.61

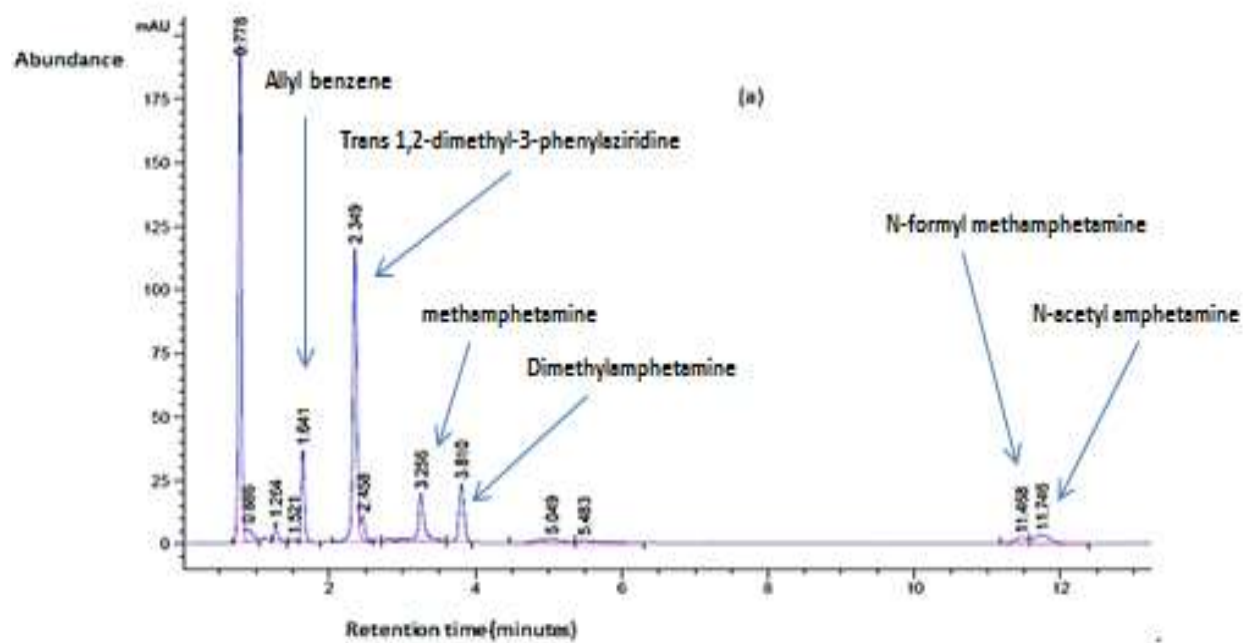


Figure 4.26 Chromatogram for pyrolysis products of methamphetamine in the mobile phase at 354°C

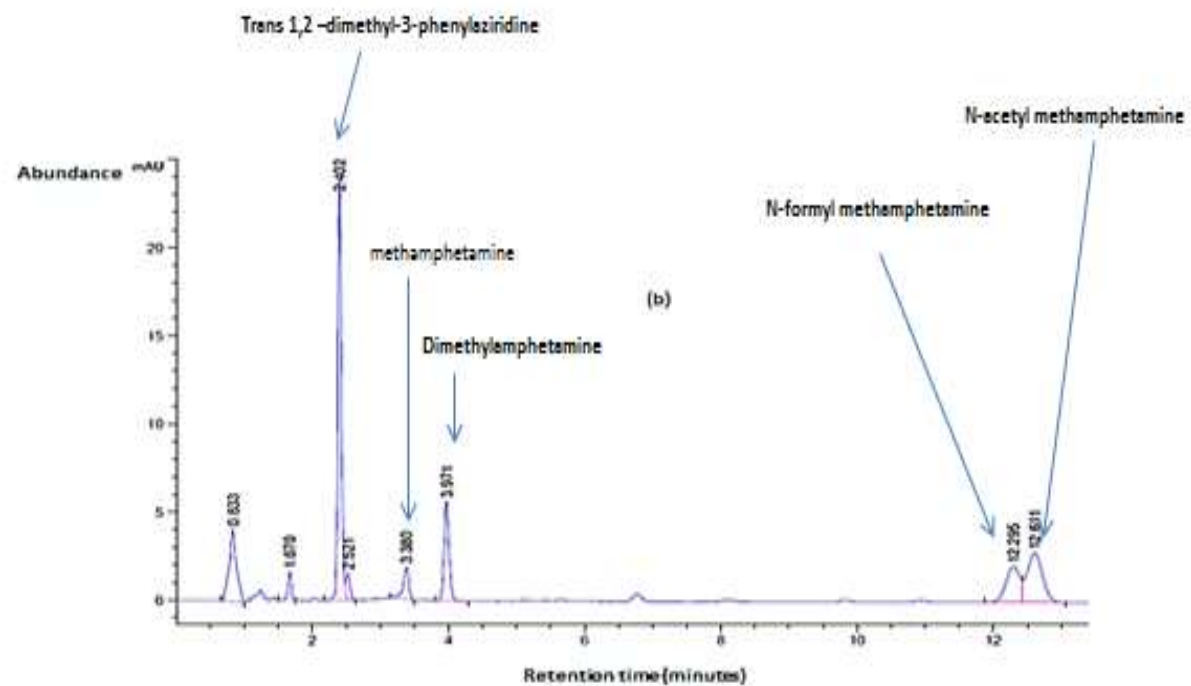


Figure 4.27 Chromatogram for pyrolysis products of methamphetamine extracted in MeOH-DCM at 354<sup>0</sup>C

The presence of all the detected products of methamphetamine was discussed in detail in the previous sections. Some products indicated the involvement of the synthetic routes of Leuckart, Emde and Nagai. Other products, di-methylamphetamine, acetyl-methamphetamine, methamphetamine and N-formylmethamphetamine are main pyrolysis products of methamphetamine via reactions of formylation, acetylation and di-methylation [169, 185].

#### **4.9.3 Evaluation of method repeatability**

The repeatability of the method for methamphetamine pyrolysis products was evaluated through calculation of %RSDs from peak areas. Peak areas for all the 6 replicate injections in tin can 1 were used to represent the within-can repeatability. The results obtained from the mobile phase are presented in table 4.16 while those from the MeOH-DCM are in table 4.17. Both results revealed %RSD values of  $\leq 5\%$ , indicating very good repeatability

The repeatability of inter-can results was also evaluated. This was obtained from %RSDs values calculated from average peak areas of pyrolysis products generated from the 6 injections in each of the 6 tin cans. The results obtained using the mobile phase are presented in table 4.18 while those for the MeOH-DCM are in table 4.19. Both results revealed %RSD values of  $\geq 5\%$ , indicating that repeatability was not achieved. Plots of peak area versus pyrolysis (figures 4.28 and 4.29) also revealed variations in the quantities of pyrolysis products.

**Table 4.16 %RSD for methamphetamine pyrolysis product vs peak area for within-can analytical repeatability extracted in the mobile phase at 354°C**

Pyrolysis product	Pyrolysis product peak area at 354°C								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
Benzaldehyde	26.24	23.98	25.38	26.24	26.24	26.33	<b>25.74</b>	<b>0.93</b>	<b>3.61</b>
allyl benzene	35.54	35.54	35.55	35.26	35.54	35.56	<b>35.50</b>	<b>0.12</b>	<b>0.33</b>
1 phenyl 1,2 propanedione	144.80	144.79	144.81	144.79	144.81	144.80	<b>144.8</b>	<b>0.008</b>	<b>0.01</b>
Trans 1,2 dimethyl phenyl aziridine	657.26	657.44	657.22	657.23	657.11	657.22	<b>657.25</b>	<b>0.12</b>	<b>0.02</b>
P2P	144.80	144.78	144.76	144.80	144.79	144.86	<b>144.80</b>	<b>0.03</b>	<b>0.02</b>
methamphetamine	144.80	144.88	144.76	144.76	144.73	144.78	<b>144.79</b>	<b>0.05</b>	<b>0.04</b>
N-formylmethamphetamine	42.56	42.54	42.57	42.65	42.57	42.56	<b>42.58</b>	<b>0.04</b>	<b>0.09</b>
N-acetylmethamphetamine	32.69	32.76	32.65	32.56	32.56	32.57	<b>32.63</b>	<b>0.08</b>	<b>0.25</b>



**Table 4.17 %RSD results for methamphetamine pyrolysis product vs peak area for within-can analytical repeatability extracted in the MeOH-DCM at 35<sup>40</sup>C**

Pyrolysis product	Pyrolysis product peak area at 354 <sup>0</sup> C								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stddev	%RSD
Allylbenzene	5.123	5.123	5.122	5.233	5.220	5.234	<b>5.18</b>	<b>0.06</b>	<b>1.13</b>
Trans 1,2 Dimethyl -3-phenylaziridine	91.370	91.370	91.33	91.36	91.35	95.360	<b>92.02</b>	<b>1.63</b>	<b>1.78</b>
P2P	5.920	5.910	5.910	5.940	5.910	5.910	<b>5.92</b>	<b>0.01</b>	<b>0.20</b>
Methamphetamine	10.371	10.344	10.365	10. 557	10.345	10.358	<b>10.36</b>	<b>0.01</b>	<b>0.12</b>
di-methyl amphetamine	29.960	29.468	29.676	29.923	29.983	29.656	<b>29.78</b>	<b>0.21</b>	<b>0.70</b>
N-formyl methamphetamine	29.123	29.123	29.121	29.124	29. 129	29.211	<b>29.14</b>	<b>0.04</b>	<b>0.14</b>
N-acetylmethamphetamine	44.263	44.362	44.331	44.232	44.452	45.213	<b>44.48</b>	<b>0.37</b>	<b>0.83</b>

**Table 4.18 %RSD results for methamphetamine pyrolysis products vs peak area for inter-can analytical repeatability extracted in the mobile phase**

Pyrolysis product	Pyrolysis product peak area at 354 <sup>0</sup> C								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
Benzaldehyde	26.243	23.901	25.384	27.973	26.260	28.271	<b>26.34</b>	<b>1.63</b>	<b>6.19</b>
allyl benzene	35.540	55.142	114.58	65.799	78.354	99.543	<b>74.83</b>	<b>29.04</b>	<b>38.81</b>
1 phenyl 1,2 propanedione	657.262	376.62	425.716	465.234	491.562	397.072	<b>468.91</b>	<b>101.54</b>	<b>21.65</b>
Trans 1,2 dimethyl phenyl aziridine	144.802	20.301	36.900	352.221	243.121	234.44	<b>171.96</b>	<b>129.18</b>	<b>75.12</b>
P2P	144.801	140.94	119.61	410. 543	210.322	103.367	<b>143.81</b>	<b>40.79</b>	<b>28.36</b>
methamphetamine	42.561	22.162	26.772	26.786	47.342	43.343	<b>34.83</b>	<b>10.76</b>	<b>30.90</b>
N-formylmethamphetamine	32.691	33.049	49.741	44.226	44.243	45.354	<b>41.55</b>	<b>7.02</b>	<b>16.91</b>
N-acetylmethamphetamine	26.243	23.901	25.384	27.973	26.26	28.271	<b>26.34</b>	<b>1.63</b>	<b>6.19</b>

**Table 4.19 %RSD results for methamphetamine pyrolysis product vs peak area for inter-can analytical repeatability extracted in the MeOH-DCM 90:10 at 354<sup>0</sup>C**

Pyrolysis product	Pyrolysis product peak area at 354 <sup>0</sup> C								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
Allylbenzene	5.123	19.465	6.002	15.302	6.333	7.112	<b>9.90</b>	<b>5.99</b>	<b>60.53</b>
Trans 1,2 Dimethyl -3-phenylaziridine	91.37	87.244	98.222	82.345	95.102	129.001	<b>97.21</b>	<b>16.56</b>	<b>17.03</b>
P2P	5.92	11.23	12.111	15.444	6.772	5.145	<b>9.44</b>	<b>4.12</b>	<b>43.52</b>
Methamphetamine	10.371	10.564	17.3	17.243	13.992	13.221	<b>13.78</b>	<b>3.06</b>	<b>22.18</b>
di-methyl amphetamine	29.96	37.224	56.356	32.998	76.243	36.898	<b>44.95</b>	<b>17.90</b>	<b>39.82</b>
N-formyl methamphetamine	29.123	28.098	45.434	24.339	16.993	36.843	<b>30.14</b>	<b>9.90</b>	<b>32.86</b>
N-acetylmethamphetamine	44.263	78.254	45.981	44.9	42.132	65.879	<b>53.57</b>	<b>14.91</b>	<b>27.838</b>

#### **4.10 Isolation of pyrolysis products of EFV and methamphetamine and analysis with the HPLC.**

Six replicate samples of EFV and methamphetamine were prepared by weighing 0.5g of each drug and mixing the two drugs into 6 separate tin cans. The mixture was heated to 354<sup>0C</sup> and pyrolysis products extracted according to the protocol described chapters 4.2.2.1. The products were trapped using four ACS in each of the six tin cans. Following this, the analysis sampling strategy in Chapter 4.2.2.2 was applied where two of the strips were used for GCMS analysis and two by the HPLC. One of the two strips was extracted with the MeOH-DCM 90:10 while the other with the mobile phase. Methamphetamine samples were in-house samples synthesised for research purposes

##### **4.10.1 Results and Discussions**

The results obtained (Table 4.20) (figure 4.31) for mobile phase extractions and (Table 4.21) (figure 4.32) for the MeOH-DCM 90:10 v/v revealed the presence of benzaldehyde, propenyl benzene, aziridines, methamphetamine, dimethyl amphetamine, N-acetylmethamphetamine, EFV pyrolysis product 1 and EFV. The first three products from recovered list are all impurities from chemicals used for the manufacture of starting materials for methamphetamine synthesis while methamphetamine may have been detected as the main product or as product from incomplete hydrolysis of the intermediate N-formyl methamphetamine. Dimethyl amphetamine and N-acetyl methamphetamine are main pyrolysis products of methamphetamine formed through acetylation and dimethylation reactions.

**Table 4.20 Pyrolysis products of EFV and methamphetamine extracted in the mobile phase at 354°C**

Pyrolysis Temperature ( in degrees Celsius)	HPLC methamphetamine	
	MeOH-DCM 9010 v/v	Retention time (minutes)
315	EFV product 1	1.428
	Allylbenzene	1.302
	1,2 dimethyl -3-phenylaziridine,trans	2.386
	Methamphetamine	3.318
	di-methyl amphetamine	3.858
	EFV	10.208
	N-acetylmethamphetamine	11.772
354	EFV product 1	1.428
	Allylbenzene	1.302
	1,2 dimethyl -3-phenylaziridine,trans	2.386
	Methamphetamine	3.318
	di-methyl amphetamine	3.858
	EFV	10.208
	N-acetylmethamphetamine	11.772

**Table 4.21 Pyrolysis products of EFV-methamphetamine extracted in MeOH-DCM 90:10 at 354<sup>0</sup>C**

<b>Pyrolysis Temperature ( in degrees Celsius)</b>	<b>HPLC methamphetamine</b>	
	<b>MeOH-DCM 9010 v/v</b>	<b>Retention time (minutes)</b>
315	Benzaldehyde	1.24
	EFV product 1	1.42
	Allylbenzene	1.65
	1,2 dimethyl -3-phenylaziridine,trans	2.35
	Methamphetamine	3.28
	di-methyl amphetamine	3.84
	EFV	10.20
	N-acetylmethamphetamine	11.74
354	Benzaldehyde	1.24
	EFV product 1	1.42
	Allylbenzene	1.65
	1,2 dimethyl -3-phenylaziridine,trans	2.35
	Methamphetamine	3.28
	di-methyl amphetamine	3.84
	EFV	10.20
	N-acetylmethamphetamine	11.74

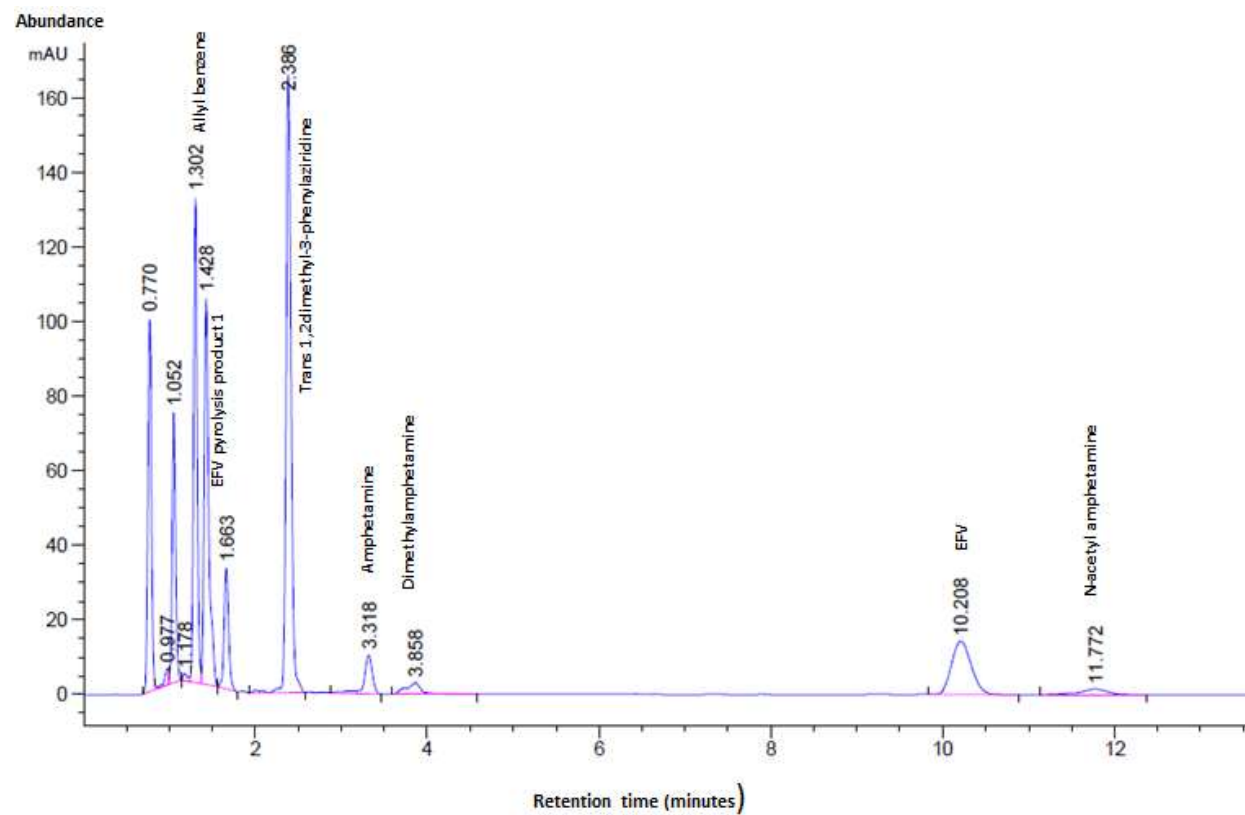


Figure 4.31 Chromatogram for pyrolysis products of EFV and methamphetamine extracted in the mobile phase at 354<sup>0</sup>C

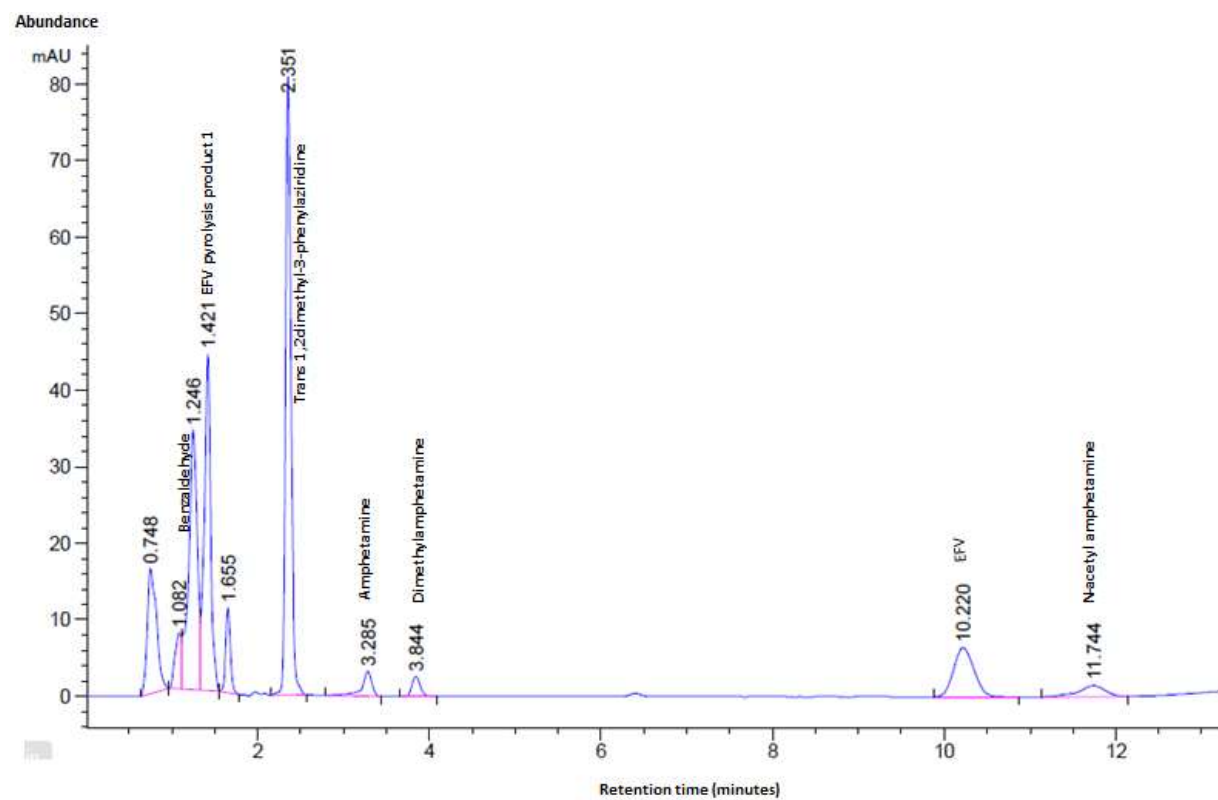


Figure 4.32 Chromatogram for pyrolysis products of EFV and methamphetamine extracted in the MeOH-DCM at 354<sup>0</sup>C



#### **4.10.2 Evaluation of the repeatability of the method for pyrolysis products of EFV-methamphetamine**

The repeatability of the method for the EFV-methamphetamine pyrolysis products was evaluated through the calculation of %RSDs of product peak areas extracted using both solvents. The peak areas for all the 6 replicates in tin can 1 were used as the overall representation of within-can repeatability. The results obtained from extractions with the mobile phase are presented in table 4.22 while those from the MeOH-DCM are in table 4.23. Both results revealed %RSD values of  $\leq 5\%$ , indicating very good repeatability

The repeatability of inter-can results was also evaluated. This was obtained from %RSDs values calculated from average peak areas of pyrolysis products generated from the 6 injections in each of the 6 tin cans ( $n=36$ ). The results obtained from the mobile phase are presented in table 4.24 while those from the MeOH-DCM are in table 4.25. Both results revealed %RSD values of  $\geq 5\%$ , indicating that repeatability was challenging. This was as expected given the conditions of the pyrolysis experiment settings.

Plots of peak area versus pyrolysis (figures 4.33 and 4.34) also revealed variations in the quantities of detected pyrolysis products. It was also discovered from these plots that a larger quantity of detected pyrolysis products was recovered from extractions involving the MeOH-DCM 90:10 solvent. Trans-1,2-dimethyl-3-phenylaziridine was found to be detected in higher amounts than other products (figure 4.35).

**Table4.22 %RSD results for EFV-methamphetamine pyrolysis product vs peak area for within-can analytical repeatability extracted in the mobile phase at 354°C**

Pyrolysis product	Pyrolysis product peak area at 354°C								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
Benzaldehyde	396.146	396.164	403.115	396.656	399.9	399.434	398.57	2.77	0.70
allylbenzene	119.43	119.43	115.743	114.904	114.86	109.543	115.65	3.66	3.17
Trans 1.2 dimethyl-3-phenylaziridine	574.711	574.712	588.443	576.876	587.432	588.981	580.43	6.92	1.19
Methamphetamine	54.565	54.564	66.755	56.987	59.138	58.321	87.10	4.51	5.13
di-methyl amphetamine	52.586	52.586	56.876	56.641	56.665	57.331	55.45	2.23	4.02
EFV1	419.468	419.468	374.031	396.373	387.35	426.37	403.84	21.03	5.21
EFV	141.696	141.696	134.765	145.745	147.61	145.705	142.87	4.63	3.24

**Table 4.23 %RSD results for EFV-methamphetamine pyrolysis product vs peak area for within-can analytical repeatability extracted in MeOH-DCM 90:10 at 354°C**

Pyrolysis product	Pyrolysis product peak area at 354°C								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
benzaldehyde	236.44	236.44	236.421	236.443	236.411	235.411	236.261	0.42	0.18
allylbenzene	42.901	42.94	42.91	42.91	42.902	42.931	42.92	0.02	0.04
Trans 1,2 dimethyl-3-phenyl aziridine	351.22	351.22	351.21	352.11	351.2	351.22	351.36	0.37	0.10
Methamphetamine	26.021	26.18	26.011	26.181	26.111	26.21	26.12	0.09	0.33
di-methyl amphetamine	17.24	17.24	17.221	17.211	17.221	17.221	17.23	0.01	0.07
EFV 1	226.373	226.373	226.37	226.65	226.374	226.37	226.42	0.11	0.05
EFV	105.751	105.66	105.75	105.751	105.751	105.752	105.74	0.04	0.44
N-acetylmethamphetamine	30.95	30.95	30.93	30.91	30.92	30.95	30.935	0.02	0.06

**Table 4.24 %RSD results for EFV-methamphetamine pyrolysis product vs peak area for inter-can analytical repeatability extracted in the mobile at 354<sup>0</sup>C**

Pyrolysis product	Pyrolysis product peak area at 354 <sup>0</sup> C								
	Can 1	Can 2	can 3	can 4	can 5	can 6	Average	Stddev	%RSD
benzaldehyde	396.146	396.164	403.115	236.436	329.9	236.440	333.03	79.44	23.85
allylbenzene	119.43	119.43	115.743	42.904	114.86	99.543	101.99	29.87	29.29
Trans 1,2 dimethyl-3-phenylaziridine	574.711	574.712	708.572	351.267	901.754	351.223	577.04	211.10	36.74
Methamphetamine	54.565	54.564	66.755	26.095	99.138	26.011	54.52	27.43	50.315
di-methyl amphetamine	52.586	52.586	38.496	17.241	73.216	17.243	41.89	22.08	52.69
EFV 1	419.468	419.468	374.031	226.373	317.35	226.370	330.51	88.10	26.92
EFV	141.696	141.696	227.112	105.745	217.094	105.745	156.51	53.38	34.12

**Table 4.25 %RSD results for EFV-methamphetamine pyrolysis product vs peak area for inter-can analytical repeatability extracted in MeOH-DCM 90:10 at 354°C**

Pyrolysis product	Pyrolysis product peak area at 354°C								
	Can 1	Can 2	Can 3	Can 4	Can 5	can 6	Average	Stdev	%RSD
benzaldehyde	236.44	236.44	236.421	236.443	256.4	115.221	219.53	47.23	21.51
allylbenzene	42.901	42.94	42.91	22.91	52.902	42.931	41.249	9.83	23.83
Trans 1,2 dimethyl-3-phenyl aziridine	351.22	351.22	345.781	352.11	267.09	351.22	351.36	34.05	9.69
Methamphetamine	26.021	26.18	58.023	26.181	26.111	15.56.21	26.12	14.27	54.62
di-methyl amphetamine	17.24	17.24	17.221	39.711	17.221	23.201	17.23	9.01	52.32
EFV 1	226.373	226.373	305.671	226.65	226.374	156.364	226.42	47.27	20.88
EFV	105.751	105.66	115.75	95.751	105.751	115.752	105.74	7.531	7.12
N-acetylmethamphetamine	30.95	30.95	29.113	36.231	30.92	91.95	30.935	24.74	79.98

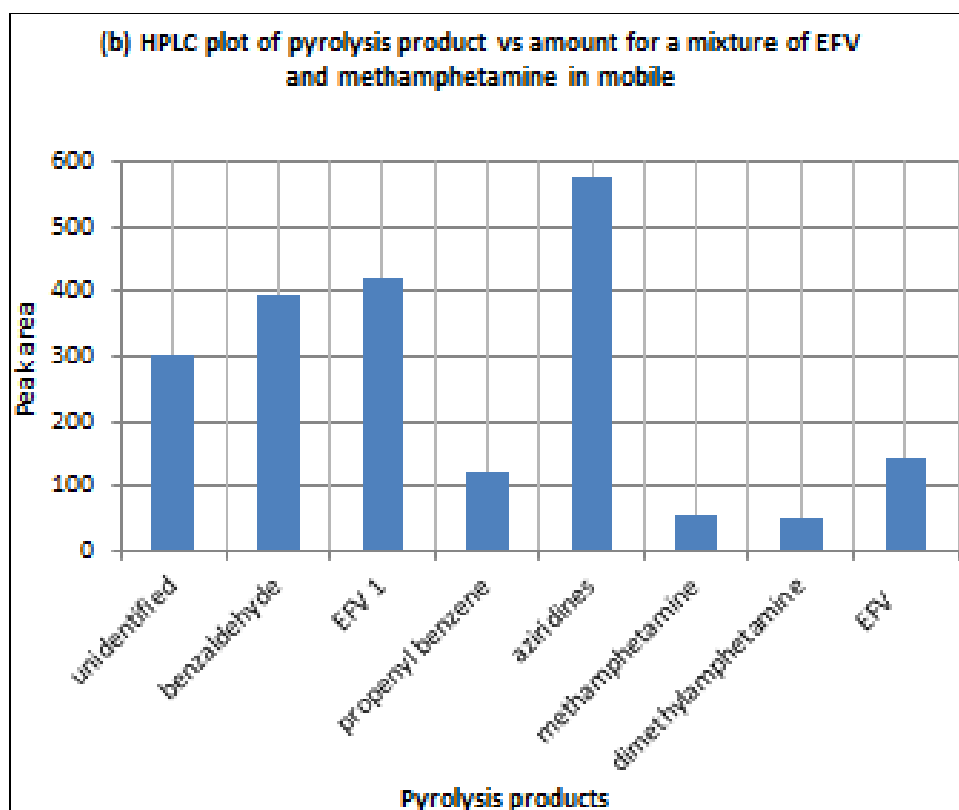
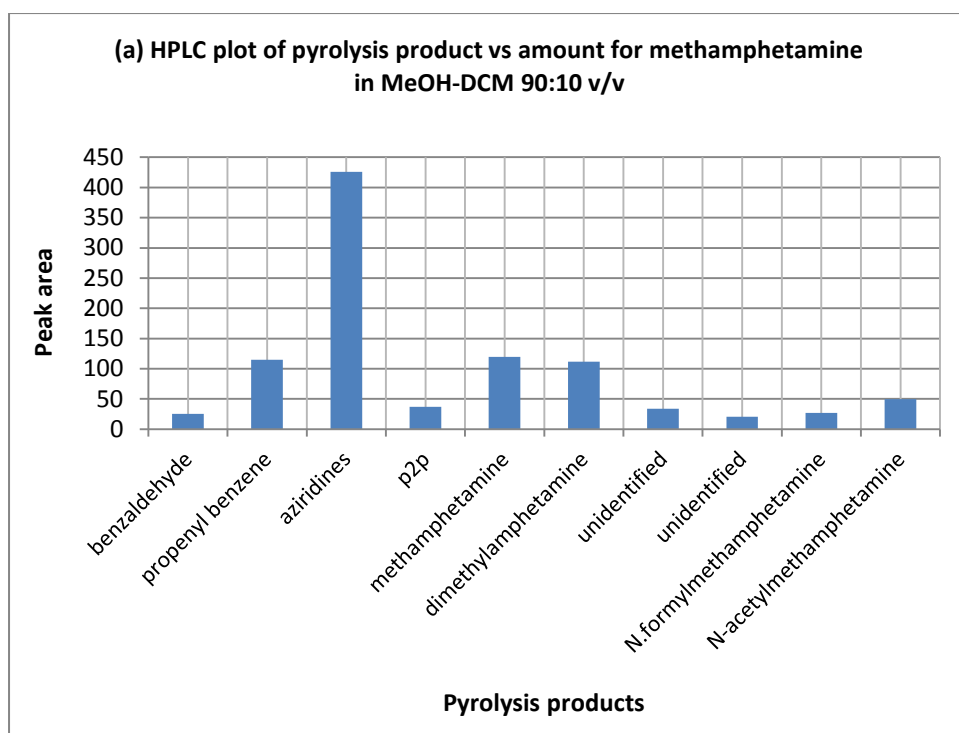


Figure 4.35 Pyrolysis products of methamphetamine detected when it was heated (a) alone and extracted in the mobile phase (b) heated with EFV and extracted with the mobile phase

From figure 4.35 it can be seen that a larger quantity of pyrolysis products was recovered when methamphetamine was heated in the presence of the EFV. It was also noted that a smaller number of products were recovered from the same extraction. The presence of both drugs in the pyrolysis mixture did not affect either drug.

#### **4.11 Isolation of pyrolysis products of amphetamine and analysis with the GCMS**

##### **4.11.1 Sample preparation**

Six replicate amphetamine samples were prepared following the protocol described in section 4.2.2.1. The samples were sourced from finished forensic cases. The amphetamine samples were first homogenised by crushing them into fine powder using a mortar and pestle. 0.5g of the powdered material was weighed into 6 separate tin cans. Each tin can was covered with a lid, heated to the desired temperature and removed from the hot plate. The covering lid was replaced with the one with prepared CAS strips (section 4.2.2.1) and incubated for 16 hours at 80°C to trap the pyrolysis products. The products were trapped using four ACS in each of the six tin cans. Following this, the analysis sampling strategy in section 4.2.2.2 was applied. For analysis with the GCMS, one of the four ACS strips was extracted with MeOH-DCM 90:10 and the other with MeOH-DCM 50:50.

##### **4.11.2. Results and Discussions**

The results obtained (table 4.26) show the detection of different amphetamine synthetic route impurities. They revealed the presence of known intermediate products from various chemical reactions involved during synthesis of the amphetamine samples; they included N-formylamphetamine and N-acetylamphetamine. N-formylamphetamine may suggest the involvement of the Leuckart amphetamine synthesis route, as illustrated on figure (4.36) while N – acetyl amphetamine while may be indicative of the use of the reductive amination synthetic route (figure 4.37). Amphetamine may have been detected as the main product or as a breakdown product from the intermediate product N-formylamphetamine. Typical chromatograms obtained from the pyrolysis of

amphetamine are displayed in figures 4.38 for products extracted with the MeOH-DCM 90:10 and figure 4.41 for the MeOH-DCM 50:50. The four main detected pyrolysis products of amphetamine are displayed in figure 4.42.



**Table 4.26 Pyrolysis products of amphetamine extracted in the two MeOH-DCM solvent ratios solvents at three different temperatures of 315, 354 and 398<sup>0</sup>C**

ACS extracting solvent	Pyrolysis Temperature ( <sup>0</sup> C)		
	315	354	394
<b>MeOH:DCM 50:50</b>	Amphetamine N-Formylamphetamine N-Acetylamphetamine	N-Formylmethamphetamine N-Acetylamphetamine N-Formylamphetamine 2-Nitro-1-phenylpropene 1-phenyl -1,2 – Propanedione alpha-methyl- Benzeneethanol Benzaldehyde 5-Hydroxymethyl-2- furancarboxaldehyde 5 hydroxymethyl furfural	N-Acetylamphetamine N-Formylamphetamine [1,3]Diazepan-2-4-dione 1-phenyl -1,2 – Propanedione alpha-methyl-Benzeneethanol Benzaldehyde 2-Propylbenzene 1-Propylbenzene
<b>MeOH:DCM 90:10</b>	Amphetamine 1,2-Propanediol, 1 –phenyl Benzaldehyde	n- Acetylamphetamine N-Formylamphetamine Benzaldehyde alpha-methyl- Benzeneethanol	N-Acetylamphetamine N-Formylamphetamine Benzyl methyl ketone Propylbenzene Benzaldehyde

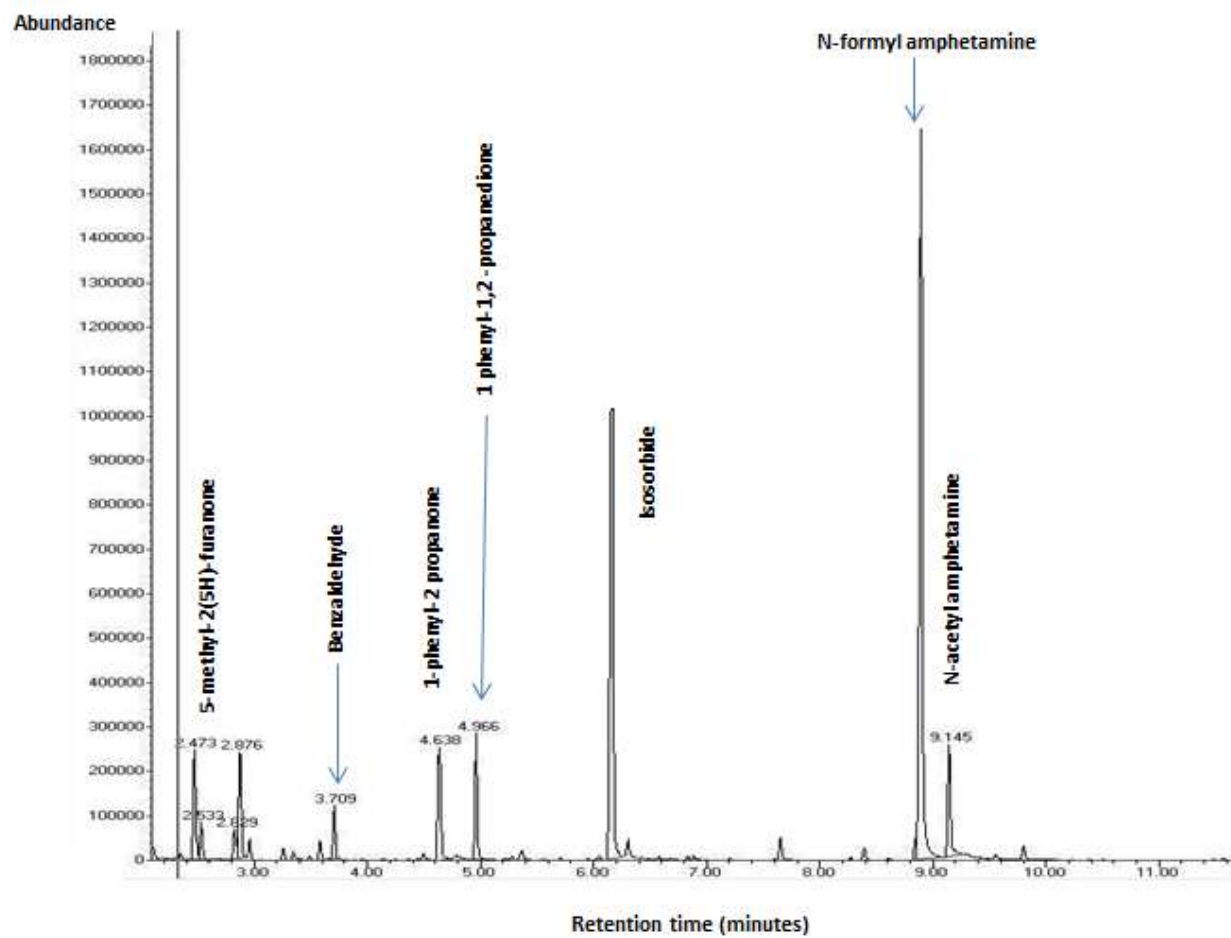


Figure 4.36 Chromatogram for pyrolysis products of amphetamine extracted in MeOH-DCM 50:50 at 354°C

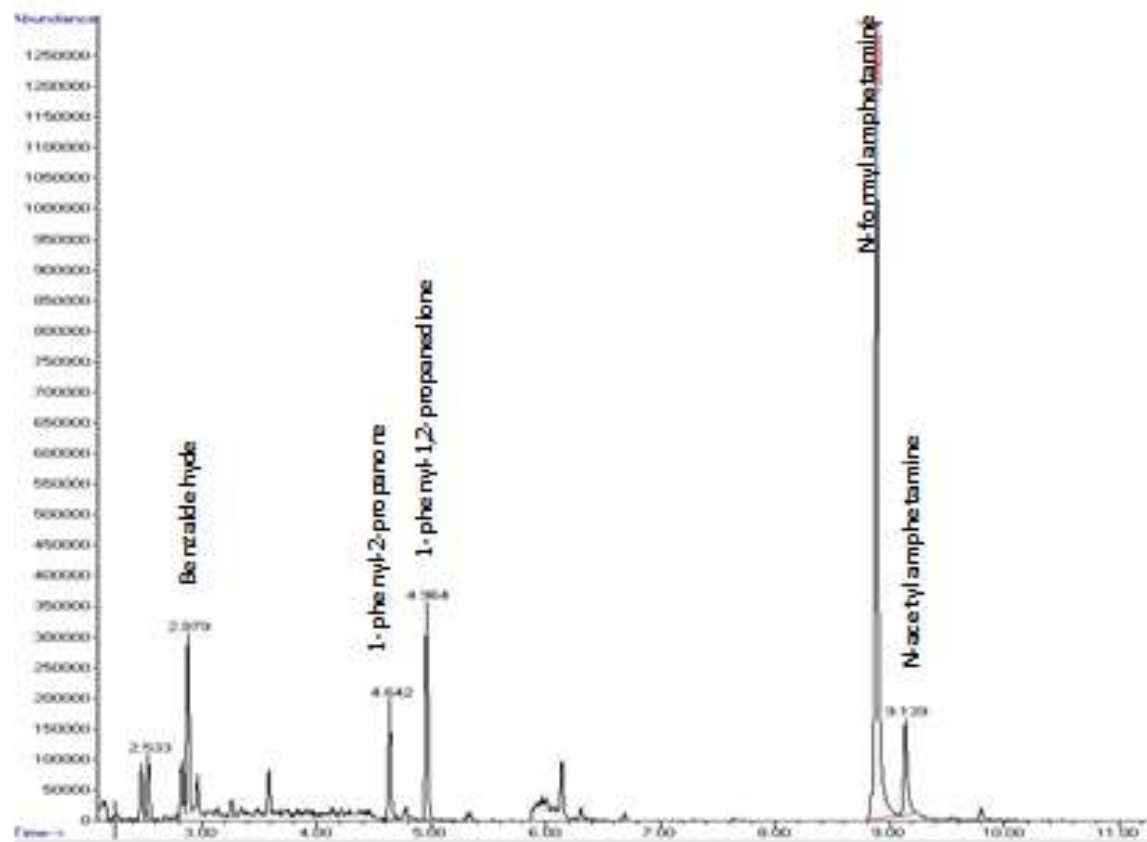


Figure 4.37 Chromatogram for pyrolysis products of amphetamine extracted in MeOH-DCM 90:10 at 354<sup>0</sup>C

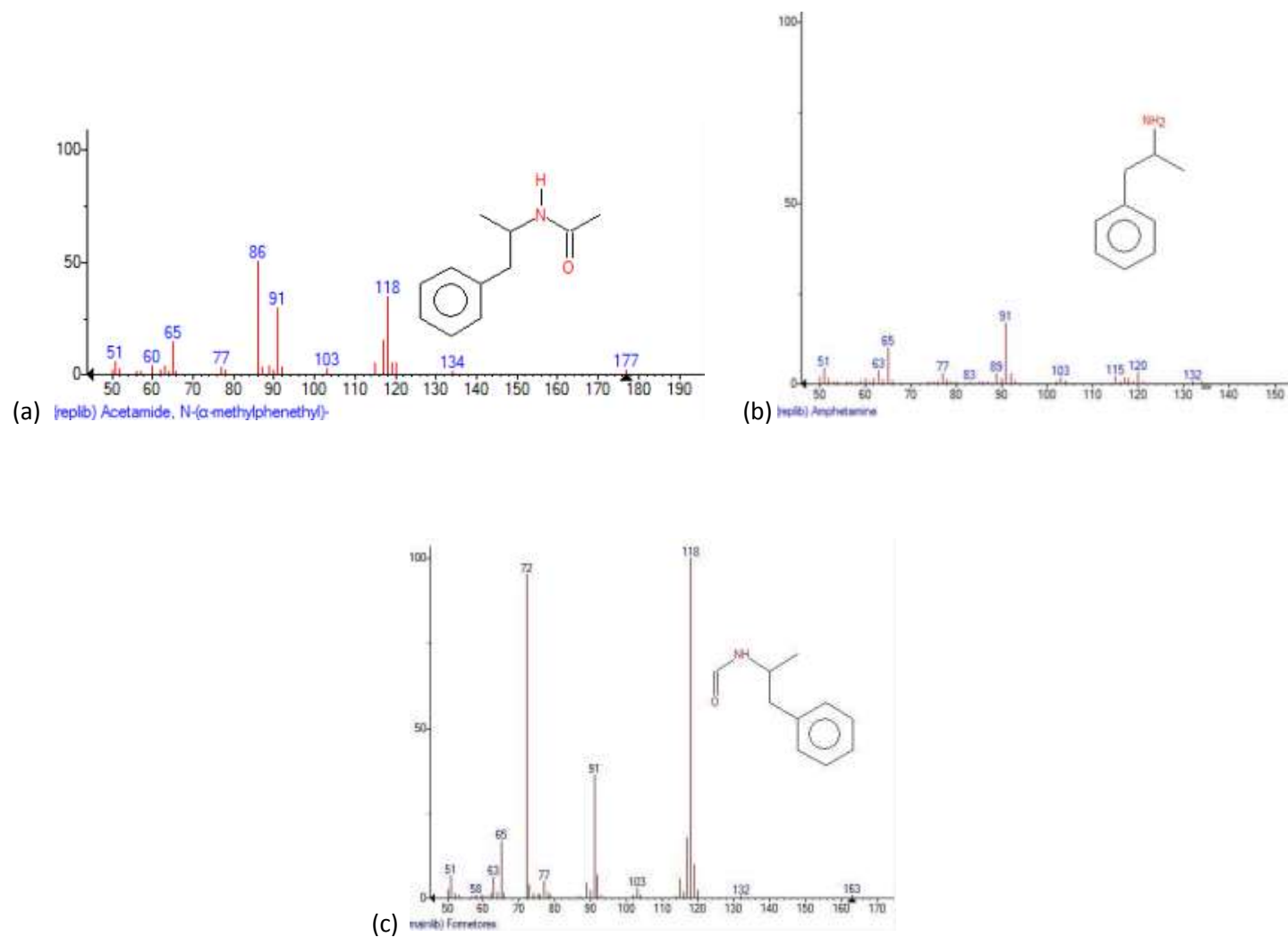
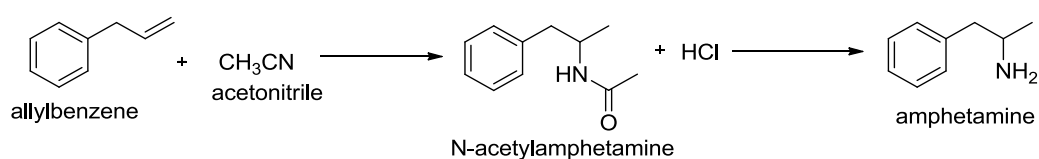


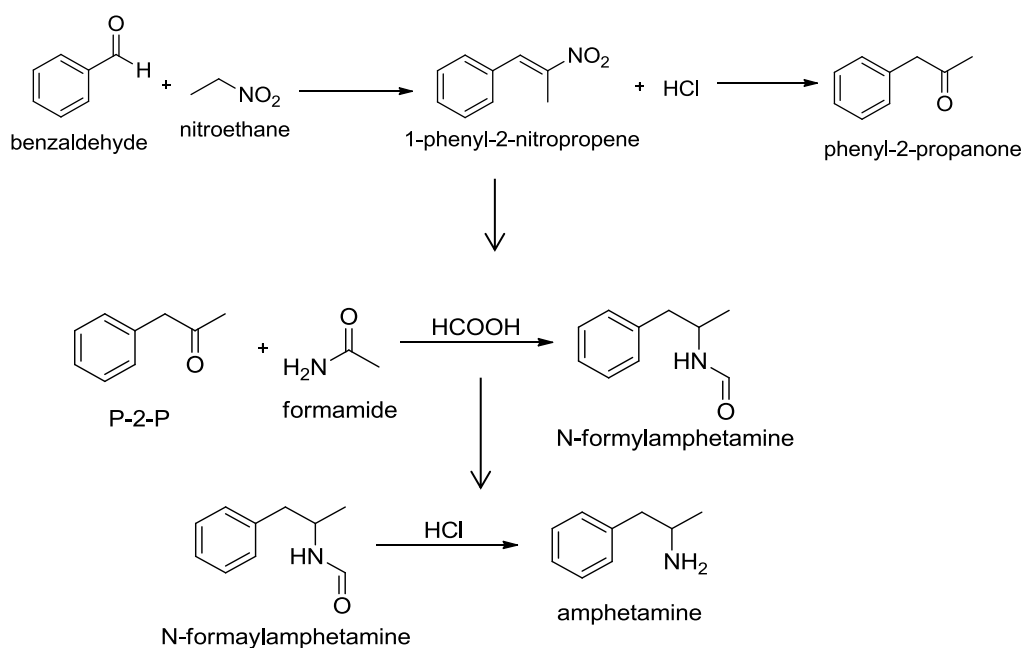
Figure 4.38 Chemical structures and mass spectra of three main pyrolysis products of amphetamine (a) N-acetylphenethylamine (b) amphetamine (c) N-formylphenethylamine

The detection of benzaldehyde is suggestive of its use as a starting material in the reductive amination synthesis of P2P [6] (figure 4.39), which itself is a starting material for amphetamine synthesis through the Leuckart route while 1-phenyl-2-nitropropene is produced as an intermediate product in the same synthetic reaction.

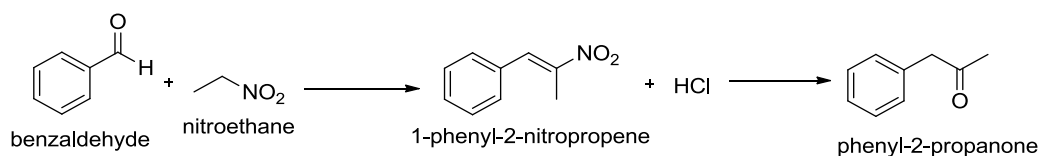
Various synthetic routes for amphetamine are displayed in figure 4.39 to 4.40 below.



**Figure 4.39 Amphetamine synthesis through the reductive amination route [169]**

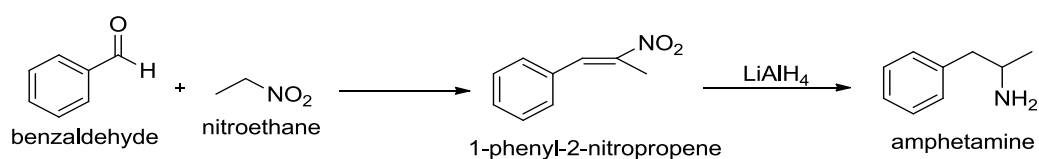


**Figure 4.40 Amphetamine synthesis through the Leuckart route [184]**



**Figure 4.41 P2P synthesis using benzaldehyde [176]**

Both chemicals can also be used without involving P2P, where the reduction of nitro-1-phenylpropene with excess lithium aluminium hydride produces amphetamine [4, 6, 24] (figure 4.41-4.42)



**Figure 4.42 Amphetamine synthesis through via reductive amination [185]**

Based on the results obtained, the possibility of the involvement of the reductive amination synthetic route seems unlikely; the detection of N-formylamphetamine in all samples and the presence of the two breakdown products of P2P (1-phenyl - 1,2-Propanedione and 1 -phenyl-1,2-Propanediol) [183] supports the use of the Leuckart synthetic route for the synthesis of the samples used. Other products detected 2-propylbenzene, 1-propylbenzene and alpha-methyl-benzeneethanol were detected. The results also revealed the presence of amphetamine, and based on this it can be said that amphetamine does not undergo complete thermal decomposition when heated to temperatures below 315<sup>o</sup>C or it could have resulted from the breakdown of the intermediate product N-forymylamphetamine.

Many methods are available for illicit amphetamine synthesis. The most common being the Leuckart reaction, favoured for its simplicity, rapidness, safety and good yield [22]. Since most chemicals used for illicit manufacture of amphetamine are controlled by law, the precursor chemicals are also manufactured in clandestine laboratories. Several impurities including benzyl methyl ketone, dibenzyl ketone, pheny-2-propanone (P2P), formic acid, formamide [169, 184, 185] and others were

observed within the pyrolysis products generated. Intermediate products also observed within the pyrolysis products of the samples such as N-formlyamphetamine and N-acetylamphetamine [169, 184] were also detected.

#### **4.11.3 Evaluation of the analytical repeatability for pyrolysis products of amphetamine**

The repeatability of the method for the production of pyrolysis products of amphetamine was evaluated to determine the analytical repeatability for desorbed samples. This was performed in two steps; the first was involved with the establishment of repeatability of results within one sampling tin (within-can repeatability) and the second was through the assessment of analytical repeatability between the 6 six tin cans. For within-can repeatability, two sets of 6 replicate samples were prepared by weighing 0.5g of amphetamine into 6 tin cans per set, these were heated and pyrolysis products trapped and extracted as per the protocol in 4.2.2.1. Each sampling tin can contained a total of 6 ACS strips. The trapped pyrolysis products from each set were desorbed using the appropriate solvent and ratio. Each resultant extract was analysed six times to facilitate the calculation of repeatability within a desorption and analysis event. Chromatographic profiles of pyrolysis products generated from each of the six replicate injections in each sample can were plotted and compared for similarities in retention time, peak shape, peak height as well as any differences in overall chemical profile.

Within-can repeatability was evaluated through %RSDs values calculated from average peak areas of pyrolysis products generated detected from the 6 replicate injections performed on each of the 6 strips in each tin can using both solvents and different ratios.

%RSD results for within-can repeatability for each pyrolysis product obtained from the 6 cans were compiled (average peak areas of pyrolysis products detected from the 6 injections in each of the 6 tin cans (n=36); their %RSDs values calculated and used to establish between-can repeatability.

#### 4.11.3.1 Analytical repeatability results

Typical chromatographic profiles of the detected pyrolysis products obtained from the 6 injections in tin can 1, (extracted using both MeOH-DCM 90:10 and MeOH-DCM 50:50 solvents at 354<sup>0</sup>C) are displayed in figures 4.43 and 4.44 (appendix A). They all show similar retention times, similar pyrolysis products and quantities. Tables 4.27 and table 4.28 (appendix B) show calculated %RSDs for tin can 1 using MeOH:DCM 90:10 and MeOH-DCM 50:50 respectively. Calculated %RSDs for the analysis were all  $\leq 5\%$ , indicating excellent repeatability.

Chromatographic profiles of pyrolysis products generated across the six different tin cans were compared. This was followed by the calculation of %RSDs across these samples. Figures 4.45 and 4.46 (appendix A) show typical chromatographic profiles of amphetamine pyrolysis products generated at 354<sup>0</sup>C for the MeOH-DCM 50:50 and 90:10 solvents respectively. Profiles revealed similar retention times but calculated %RSD values (tables 4.29 and 4.30) (appendix B) were all  $\geq 5\%$ , indicating that repeatability was considerably more challenging. Plots of pyrolysis product versus product peak area (figures 4.47 and 4.48) (appendix A) for amphetamine pyrolysis products also revealed variations in product quantities. This is expected given the nature of the pyrolysis event and that some variation was likely.



#### **4.12 Isolation of pyrolysis products of EFV in a mixture with amphetamine and analysis with the GCMS**

Six replicate samples of EFV and amphetamine were prepared by weighing 0.5g of each drug and mixing the two drugs into 6 separate tin cans. The mixture was heated to 354<sup>0</sup>C and pyrolysis products extracted according to the protocol described in chapter 4.2.2.1. The products were trapped using four ACS in each of the six tin cans. The analysis sampling strategy in Chapter 4.2.2.2 was applied where one of the four ACS strips was extracted with MeOH-DCM 90:10 and the other with MeOH-DCM 50:50. Amphetamine samples were available from completed forensic case samples

##### **4.12.1 Results and Discussions**

Results obtained (table 4.31) revealed the detection of N-acetylamphetamine, N-formylamphetamine, benzaldehyde, 1-phenyl-1,2-propanedione, isosorbide, dimethyl-benzaldehyde, alpha-methyl-benzeneethanol. The last four products are impurities from chemicals used in the manufacture of amphetamine and their detection suggest possible involvement of the Leuckart amphetamine synthetic route. N-acetylamphetamine and N-formylamphetamine are main pyrolysis products of amphetamine and can be used to assist with the determination of synthetic route. EFV and its pyrolysis product were also detected.

Typical chromatograms for pyrolysis products obtained from the pyrolysis of the mixture of the two drugs (extracted using both solvents) are displayed in figures 4.48 and 4.49.

**Table 4.31 Pyrolysis products of EFV and amphetamine extracted in the two MeOH-DCM solvent ratios solvents at 354<sup>0</sup>C**

ACS extracting solvent	Pyrolysis Temperature (°C)	
	315	354
<b>MeOH:DCM 50:50</b>	Amphetamine N-Formylamphetamine N-Acetylamphetamine	N-Acetylamphetamine N-Formylamphetamine 2-furan methanol 1-phenyl -1,2 – Propanedione alpha-methyl- Benzeneethanol Benzaldehyde 5-Hydroxymethyl-2- furancarboxaldehyde 5 hydroxymethyl furfural EFV EFV pyrolysis product 1
<b>MeOH:DCM 90:10</b>	Amphetamine 1,2-Propanediol, 1 –phenyl Benzaldehyde N- Acetylamphetamine N-Formylamphetamine 2-furan methanol	N- Acetylamphetamine N-Formylamphetamine Benzaldehyde alpha-methyl- Benzeneethanol EFV EFV pyrolysis product 1

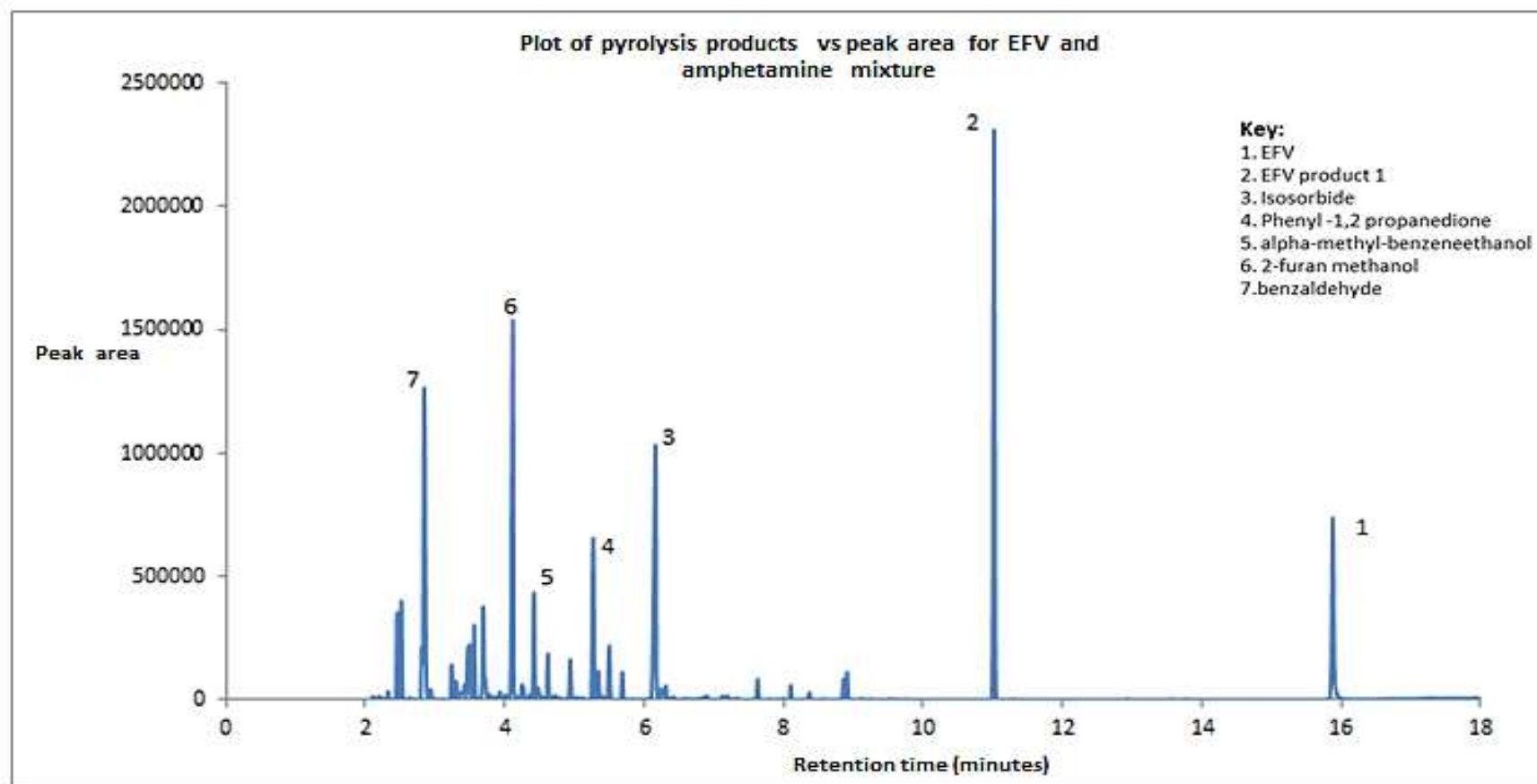


Figure 4.48 Chromatogram for pyrolysis products of EFV-amphetamine extracted in MeOH-DCM 90:10 at 354<sup>0</sup>C

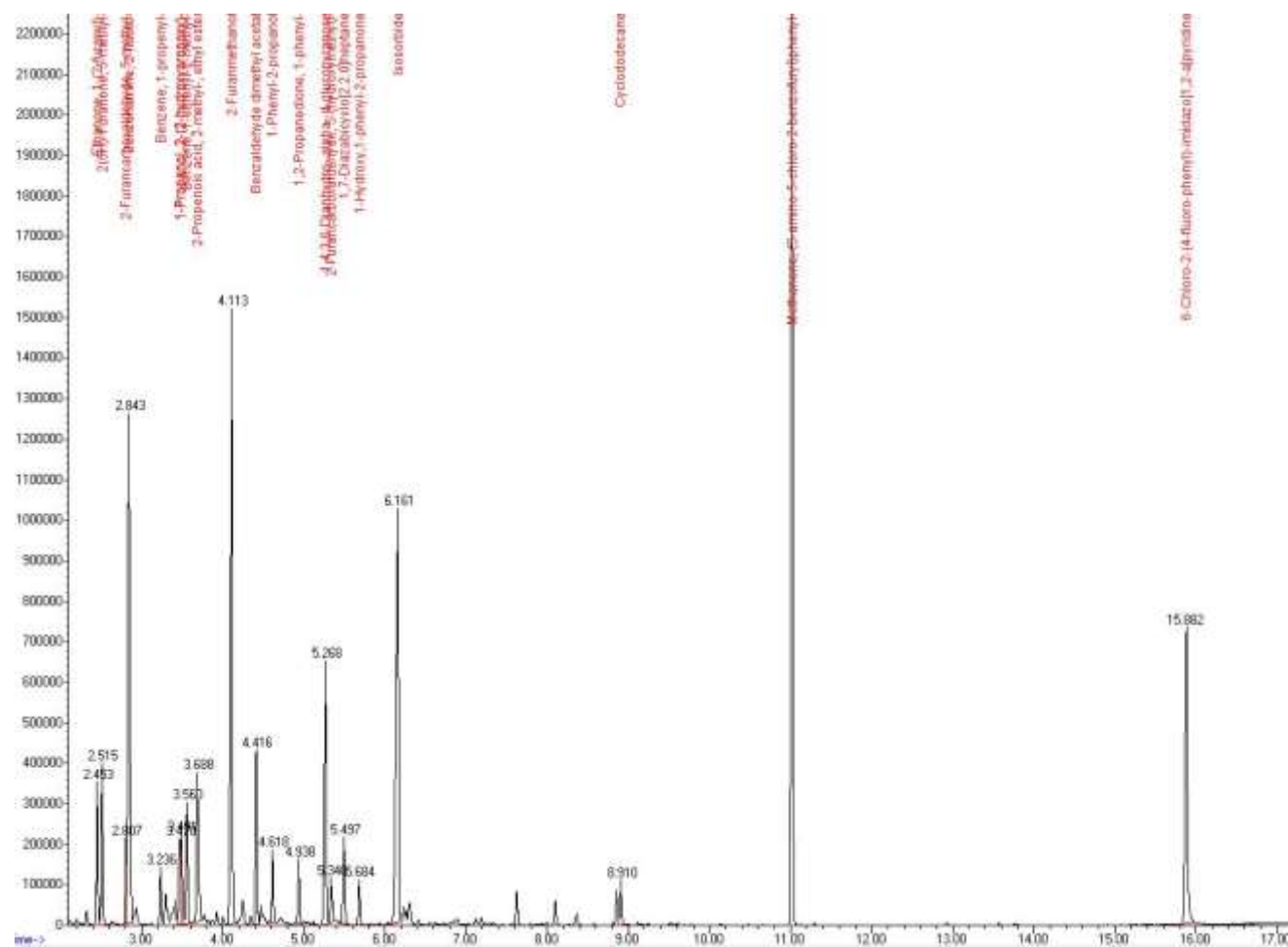


Figure 4.49 Pyrolysis products of amphetamine extracted in MeOH-DCM 50:50 at 354°C

#### **4.12.2 Analytical repeatability of the method for pyrolysis products of EFV-amphetamine**

The repeatability of the method for the production of pyrolysis products of amphetamine was evaluated to determine the analytical repeatability for desorbed samples. The first evaluation involved the establishment of repeatability of results within one sampling tin (within-can repeatability) and the second was for the repeatability of between the 6 six tin cans. Samples for within-can repeatability were prepared as previously. Pyrolysis products were extracted according to the protocol in 4.2.2.1, each sampling tin can contained a total of 6 ACS strips. The trapped pyrolysis products from each set were desorbed using the appropriate solvent and ratio. Each resultant extract was analysed six times to facilitate the calculation of repeatability within a desorption and analysis event. Chromatographic profiles of pyrolysis products generated from each of the six replicate injections in each sample can were plotted and compared for similarities in retention time, peak shape, peak height as well as any differences in overall chemical profile.

Within-can repeatability was evaluated through %RSDs values calculated from average peak areas of pyrolysis products generated detected from the 6 replicate injections performed on each of the 6 strips in each tin can using both solvents and ratios.

Average peak areas of pyrolysis products detected from the 6 injections in each of the 6 tin cans (n=36) were compiled; their %RSDs values were calculated and used to establish between-can repeatability.

##### **4.12.2.1 Repeatability results**

Typical chromatographic profiles for the detected pyrolysis products obtained from the 6 injections in tin can 1, (extracted using both MeOH-DCM 90:10 and MeOH-DCM 50:50 solvents at 354<sup>0</sup>C) are displayed in figures 4.50 and 4.51 (appendix A). They all show similar retention times, similar pyrolysis products and quantities. Table 4.32 and table 4.33 (appendix B)s how calculated %RSDs for tin can 1 using MeOH:DCM 90:10 and MeOH-DCM 50:50 respectively. Calculated %RSDs for the analysis were all  $\leq 5\%$ , indicating excellent repeatability.

Chromatographic profiles of pyrolysis products generated across the six different tin cans were also compared. This was followed by the calculation of %RSDs across these samples. Profiles revealed similar retention times but calculated %RSD values (table 4.34 and 4.35) (appendix B) were all  $\geq 5\%$ , indicating that repeatability was not achievable. Plots of pyrolysis product versus product peak area (figures 4.52 and 4.53) (appendix A) for amphetamine pyrolysis products also revealed variations in product quantities. The results were not surprising as variation would be expected to occur due the nature of the pyrolysis event.

Figure 4.52 and 4.53 (appendix A) show variations in amounts of pyrolysis products obtained from both drugs across the six cans, with the highest quantities observed for benzaldehyde, 2-furan methanol, isosorbide and EFV pyrolysis product 1. According to the results, the presence of EFV in mixture with amphetamine does not alter the expected pyrolysis patterns of amphetamine or vice versa. The number of amphetamine pyrolysis products obtained from the mixture was however less than when amphetamine was heated on its own.

### **4.13 Isolation of pyrolysis products of amphetamine and analysis with the HPLC**

#### **4.13.1 Sample preparation**

Amphetamine samples used for the HPLC analysis were the two remaining ACS strips from the four prepared earlier in section 4.3 where six replicate amphetamine samples were homogenised and prepared following the protocol described in chapters 4.2.2.1; 0.5g of the powdered material was weighed into 6 separate tin cans, heated and pyrolysis products extracted. The products were trapped using four ACS in each of the six tin cans. Following this, the analysis sampling strategy in Chapter 4.2.2.2 was applied where two of the strips were used for GCMS analysis and two by the HPLC. One of the two strips was extracted with the MeOH-DCM 90:10 while the other with the mobile phase. The samples were sourced from completed forensic work.

#### **4.13.2 Results and discussions**

Results obtained from extractions using the mobile phase are displayed in table 4.36 and figure 4.54 while figure 4.55 and Table 4.37 shows results for the MeOH-DCM 90:10. Both results revealed the detection of the same pyrolysis products being a mixture of benzaldehyde, furan methanol, P2P, and dimethyl amphetamine and amphetamine. These have been discussed in the previous section where they were discovered to be chemicals used as starting materials in the different synthetic routes mentioned earlier or intermediate products from chemical reactions involved.

**Table 4.36 Pyrolysis products of amphetamine extracted in the mobile phase at 354°C**

Pyrolysis Temperature ( in degrees Celsius)	HPLC methamphetamine	
	Mobile phase 25Mm buffer-ACN (53:47)	Retention time (minutes)
315	Benzaldehyde Furan methanol P2P Dimethyl amphetamine	1.048 1.448 2.473 3.479
354	Benzaldehyde Furan methanol P2P Dimethyl amphetamine	1.048 1.448 2.473 3.479
358	Benzaldehyde Furan methanol P2P Dimethyl amphetamine	1.048 1.448 2.473 3.479



Table 4.37 Pyrolysis products of amphetamine extracted in the MeOH-DCM 90:10 at 354<sup>0</sup>C

Pyrolysis Temperature ( in degrees Celsius)	HPLC methamphetamine	
	Mobile phase 25Mm buffer-ACN (53:47)	Retention time (minutes)
315	Benzaldehyde	1.048
	2-Furan methanol	1.448
	P2P	2.473
	dimethyl amphetamine	3.479
	N-formyl amphetamine	11.631
	N-acetylamphetamine	11.922
354	Benzaldehyde	1.048
	2-Furan methanol	1.448
	P2P	2.473
	dimethyl amphetamine	3.479
	N-formyl amphetamine	11.631
	N-acetylamphetamine	11.922
358	Benzaldehyde	1.048
	2-Furan methanol	1.448
	P2P	2.473
	dimethyl amphetamine	3.479
	N-formyl amphetamine	11.631
	N-acetylamphetamine	11.922

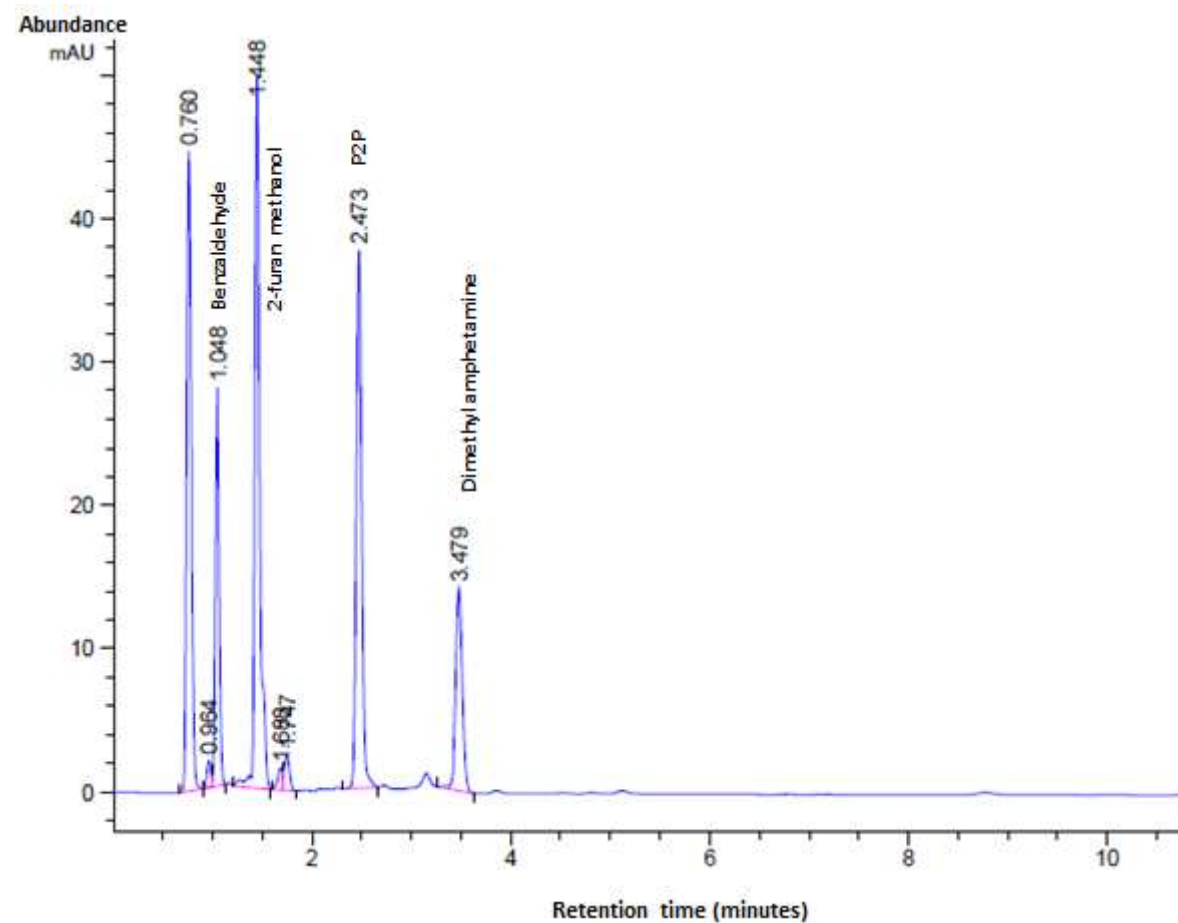


Figure 4.54 Chromatogram for pyrolysis products of amphetamine extracted in the mobile phase at 54°C

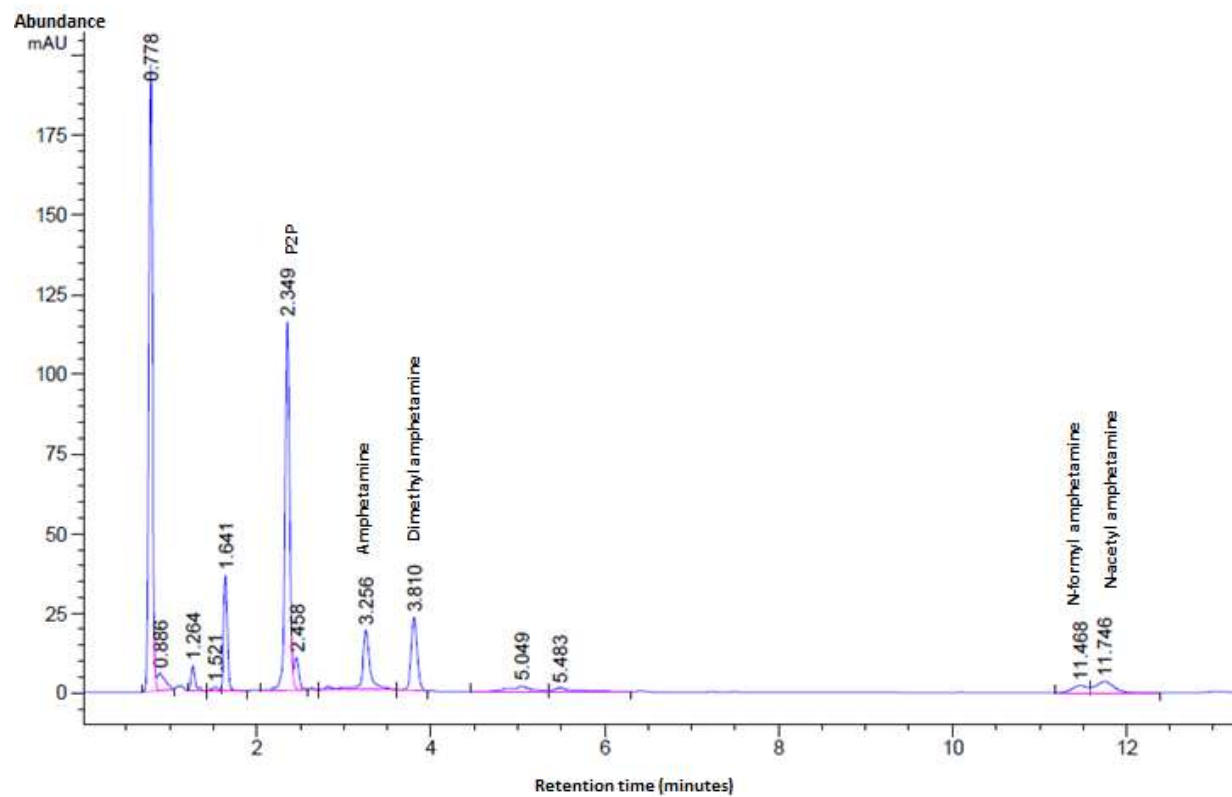


Figure 4.55 Chromatogram for pyrolysis products of amphetamine extracted in the MeOH-DCM 90:10 at 354<sup>0</sup>C

The presence of all the detected pyrolysis products of amphetamine was discussed in detail in the previous sections. The presence of some products indicates the involvement of the synthetic routes of Leuckart or reductive amination synthesis reactions, where products like dimethylamphetamine, Acetyl-methamphetamine, amphetamine and N-formylamphetamine are main pyrolysis products of amphetamine via reactions of formylation, acetylation and di-methylation [169]

#### **4.13.3 Evaluation of analytical repeatability of the method for amphetamine pyrolysis products**

The repeatability of the method for amphetamine pyrolysis products was evaluated through the calculation of %RSDs from peak areas of detected pyrolysis products. Peak areas for all the 6 replicate injections in tin can 1 were used to represent the within-can repeatability. The results obtained from the mobile phase are presented in table 4.38 while those from the MeOH-DCM are in table 4.39. Both results revealed %RSD values of  $\leq 5\%$ , indicating very good repeatability

The analytical repeatability of inter-can results was also evaluated. This was performed through the use of %RSDs values calculated from average peak areas of pyrolysis products generated from the 6 injections in each of the 6 tin cans ( $n=36$ ). The results for the mobile phase are presented in table 4.40 while those for the MeOH-DCM are in table 4.41 (appendix B). Both results revealed %RSD values of  $\geq 5\%$ , indicating that repeatability not achievable. Plots of peak area versus pyrolysis (figures 4.56 and 4.57) (appendix B) also revealed variations in the quantities of pyrolysis products.

#### **4.14 Isolation of pyrolysis products of EFV in its mixture with amphetamine and analysis with the HPLC.**

Amphetamine samples used for the HPLC analysis were the two remaining ACS strips from the four prepared earlier in section 4.3 where six replicate amphetamine samples were homogenised and prepared following the protocol described in chapters 4.2.2.1; 0.5g of the powdered material was weighed into 6 separate tin cans, heated and pyrolysis products extracted. The products were trapped using four ACS in each of the six tin cans. Following this, the analysis sampling strategy in Chapter 4.2.2.2 was applied where two of the strips were used for GCMS analysis and two by the HPLC. One of the two strips was extracted with the MeOH-DCM 90:10 while the other with the mobile phase. The samples were sourced from completed forensic work.

##### **4.14.1 Results and Discussions**

The results obtained (Table 4.42) (figure 4.58a) for mobile phase extractions and (Table 4.43) (figure 4.58b) for the MeOH-DCM 90:10 v/v revealed the presence of EFV pyrolysis product 1, EFV, benzaldehyde, dimethylamphetamine, P2P and 2-Furan methanol and other amphetamine products which could not be identified because they didn't correlate with the reference material used.

**Table 4.42 Pyrolysis products from a mixture of EFV and amphetamine extracted in the mobile phase**

Pyrolysis Temperature ( in degrees Celsius)	HPLC EFV and amphetamine	
	Mobile phase 25Mm buffer-ACN (53:47)	Retention time (minutes)
354	EFV pyrolysis product 1 EFV Benzaldehyde Dimethylamphetamine P2P 2-furan methanol	1.429 10.369 1.052, 2.392 and 3.33

**Table 4.43 Pyrolysis products from a mixture of EFV and amphetamine extracted in the MeOH-DCM 90:10**

Pyrolysis Temperature ( in degrees Celsius)	HPLC EFV and amphetamine	
	DCM-MeOH 90:10	Retention time (minutes)
354	EFV pyrolysis product 1 EFV Benzaldehyde Dimethylamphetamine P2P 2-furan methanol N-acetylamphetamine	1.426 10.284 1.299, 1.496, 2.30 and 3.310

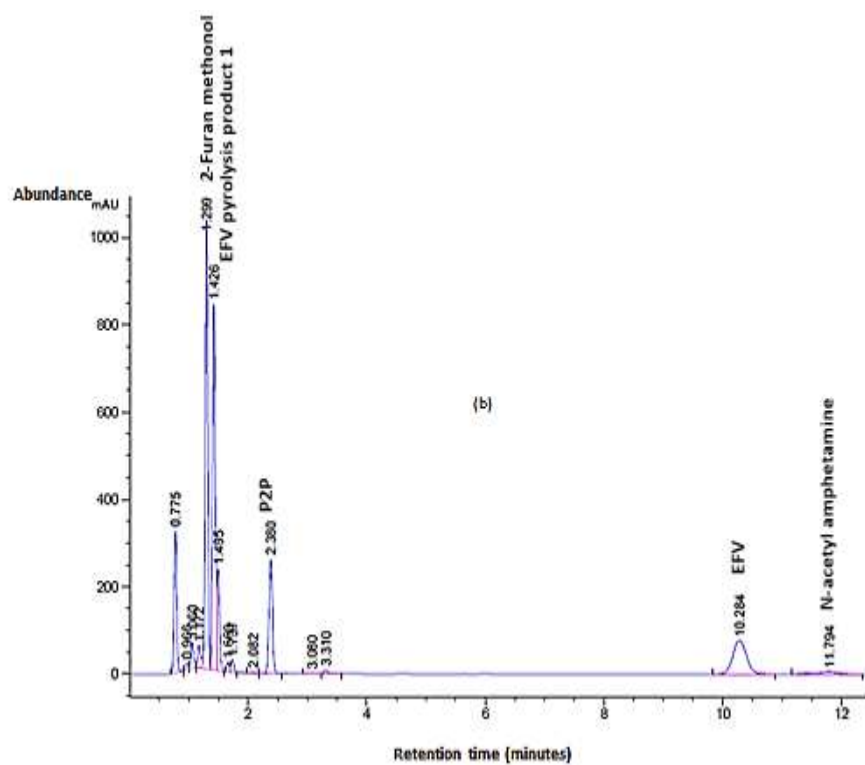
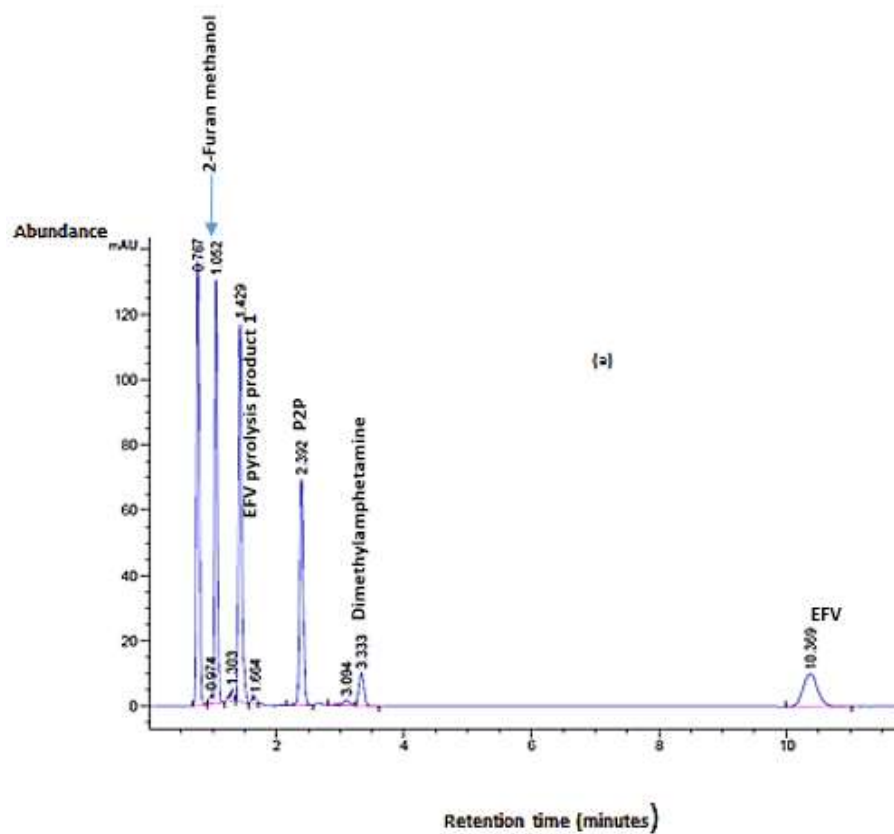


Figure 4.58 Chromatograms for pyrolysis products of amphetamine extracted in the (a) mobile phase and (b) MeOH-DCM 90:10

The presence of all the detected pyrolysis products of amphetamine was discussed in detail in the previous sections. The presence of some products indicates the involvement of the synthetic routes of Leuckart or reductive amination synthesis reactions, where products like dimethylamphetamine, Acetyl-methamphetamine, amphetamine and N-formylamphetamine are main pyrolysis products of amphetamine via reactions of formylation, acetylation and di-methylation [169, 185].

#### **4.14.2 Evaluation of analytical repeatability of the method for amphetamine pyrolysis products**

The repeatability of the method for amphetamine pyrolysis products was evaluated through the calculation of %RSDs from peak areas of detected pyrolysis products. Peak areas for all the 6 replicate injections in tin can 1 were used to represent the within-can repeatability. The results obtained from the mobile phase are presented in table 4.44 while those from the MeOH-DCM are in table 4.45 (appendix B). Both results revealed %RSD values of  $\leq 5\%$ , indicating very good repeatability

The analytical repeatability of inter-can results was also evaluated. This was performed through the use of %RSDs values calculated from average peak areas of pyrolysis products generated from the 6 injections in each of the 6 tin cans ( $n=36$ ). The results for the mobile phase are presented in table 4.46 while those for the MeOH-DCM are in table 4.47 (appendix B). Both results revealed %RSD values of  $\geq 5\%$ , indicating that repeatability not achievable. Plots of peak area versus pyrolysis (figures 4.59 and 4.60) (appendix A) also revealed variations in the quantities of pyrolysis products.



#### **4.14.2.1 Analytical repeatability results**

The results obtained from the analysis of pyrolysis products of amphetamine from a mixture of the two drugs revealed that repeatability is easily achieved for multiple single injections from ACS strips within one tin can and within one strip, indicating overall stability of the pyrolysis to analysis method. As expected the repeatability of analysis between sample cans varies much more widely. One very useful result obtained, however is that the nature of the pyrolysis mixtures did not vary significantly within a specific temperature solvent ratio mix and this provides a considerable confidence to the methodology.

It was observed that the presence of EFV in the mixture did not interfere with the pyrolysis of amphetamine; however the EFV was observed to have remained stable and did not undergo any transformation.

#### 4.15 Summary of the pyrolysis of EFV and ATS drugs.

The pyrolysis of EFV revealed the presence of only one pyrolysis product. The product was detected at 11.09 minutes with main four ions with  $m/z$  270, 245, 235, 201 and 167 with the GCMS technique and at 1.431 minutes with the HPLC mobile phase and 1.423 minutes with the MeOH-DCM 90:10 using the HPLC. A possible chemical structure of the EFV pyrolysis was proposed together with those of its main molecular ions. The pyrolysis of amphetamine type stimulant drugs revealed the formation of pyrolysis products through typical reactions of N-acetylation, N-formylation, dimethylation, N-methylation. In addition to the products formed from the reactions listed above, the pyrolysis of the ATS drugs (methamphetamine and amphetamine) revealed the presence of chemical used as starting materials and by-products of chemical reactions used during the various synthetic processes. Some of these products were used to determine possible synthetic routes for the manufacture of both methamphetamine and amphetamine samples used for this research. The results obtained from the pyrolysis of a mixture of EFV and the two ATS type drugs revealed that none of the three drugs interferes with the pyrolysis of another and that the quantities of the products produced will vary. The conclusion drawn from the pyrolysis of a mixture of these drugs was that when either of the two ATS drugs is smoked for abuse in its mixture with EFV, the inhaled pyrolysis products will likely comprise of products from both drugs.

## **CHAPTER 5: EFFECTS OF EFAVIRENZ ON THE SEMI-SYNTHETIC DRUGS ALLEGED TO BE USED IN THE NYAOPE MIXTURE.**

### **5.1 Isolation of pyrolysis products of heroin and analysis with the GCMS**

#### **5.1.1 Sample preparation**

Six replicate heroin samples were prepared following the protocol described in section 4.2.2.1. The samples were recovered from forensic case work samples. They were first homogenised by crushing them into fine powder using a mortar and pestle. 0.5g of the powdered material was weighed into 6 separate tin cans. Each tin can was covered with a lid, heated to the desired temperature and removed from the hot plate. The covering lid was replaced with the one with prepared CAS strips (section 4.2.2.1) and incubated for 16 hours at 80<sup>0</sup>C to trap the pyrolysis products. The products were trapped using four ACS in each of the six tin cans. Following this, the analysis sampling strategy in section 4.2.2.2 was applied. For GCMS analysis one of the four ACS strips was extracted with MeOH-DCM 90:10 and the other with MeOH-DCM 50:50.

#### **5.1.2 Results and discussions**

Results obtained from samples extracted with the two different solvents ratios are displayed in table 5.1 and they revealed the presence of papaverine, noscapine, diacetylmorphine, 6-monoacetylmorphine, morphine, caffeine, diacetamate, meconin and paracetamol. Typical chromatograms of pyrolysis products obtained from the pyrolysis of heroin at 354<sup>0</sup>C (extracted using MeOH-DCM 90:10 and MeOH-DCM 50:50) are displayed in figure 5.1 and 5.2 respectively. Detected pyrolysis products in both cases have been reported as alkaloids of opium, cutting agents and breakdown products of some of the main alkaloids. Heroin street samples are known to contain morphine and other alkaloids from the opium plant; these include codeine, thebaine, noscapine, and papaverine [186-188]]. This therefore explains the presence of papaverine and noscapine in the analysed samples. Meconin and hydrocortanine were detected as breakdown of noscapine [189]. Paracetamol and caffeine are common cutting agents for street heroin [190]

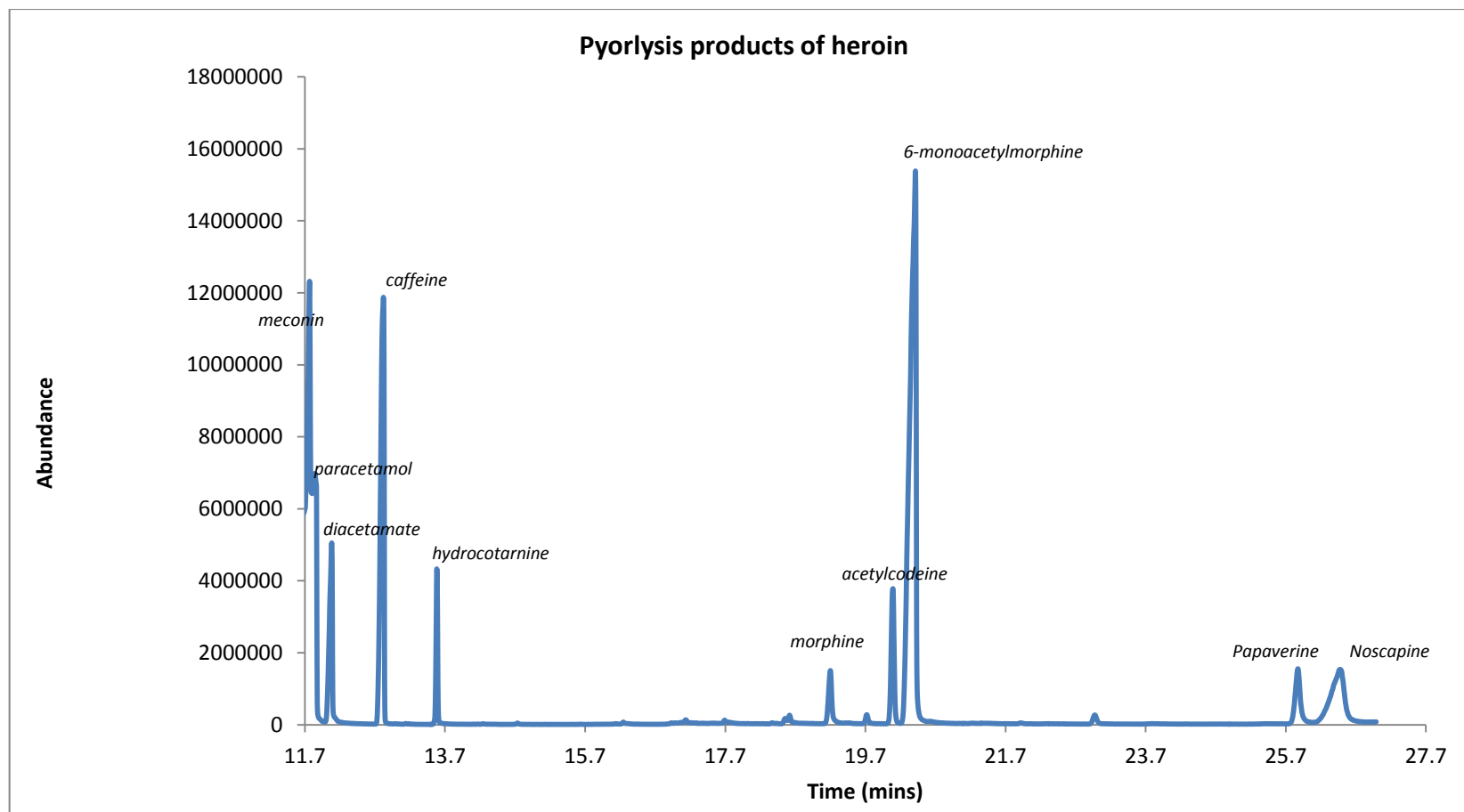
and their presence in the samples suggests they were used for such purposes. Noscapine has also been implicated as a cutting agent for heroin [190]; especially where it was detected in larger quantities than normal.

Codeine can present in the extract during the extraction of morphine, and as a result will be acetylated to acetyl codeine. This therefore explains why it was detected amongst the pyrolysis products for this experiment.

Figure 5.3 shows different chemical transformations/reactions of diacetylmorphine.

**Table 5.1 Pyrolysis products of heroin at different temperatures of 315, 354 and 398<sup>o</sup>C**

Pyrolysis Temperature ( in <sup>o</sup> C)	GCMS heroin ACS	
	DCM:MeOH 50:50	DCM:MeOH 10:90
315	acetaminophen Meconin Morphine Acetyl codeine 6 Mono acetyl morphine papaverine	acetaminophen Meconin Morphine Acetyl codeine 6 Mono acetyl morphine papaverine
354	acetaminophen Meconin lactone diacetamate caffeine hydrocortamine Acetyl codeine 6-monocaetyl morphine diacetylmorphine papaverine	acetaminophen Meconin lactone diacetamate caffeine hydrocortamine Acetyl codeine 6-monocaetyl morphine diacetylmorphine papaverine noscaphine
394	acetaminophen Meconin lactone diacetamate caffeine hydrocatamine EFV Acetyl codeine 6-monocaetyl morphine diacetylmorphine	acetaminophen Meconin lactone diacetamate caffeine hydrocatamine EFV Acetyl codeine 6-monocaetyl morphine diacetylmorphine



**Figure 5.1 Chromatogram of pyrolysis products of heroin extracted in MeOH-DCM 90:10**

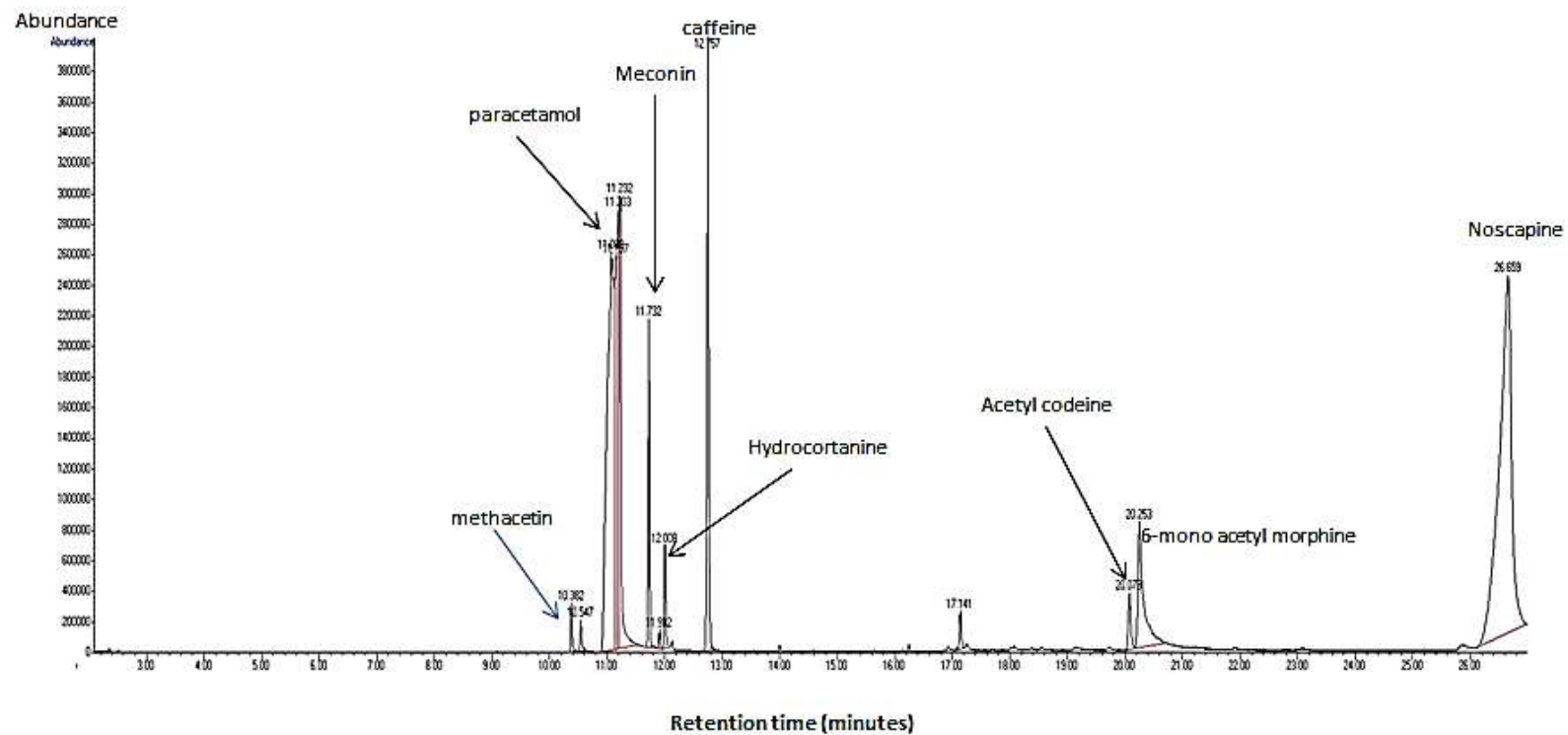


Figure 5.2 Pyrolysis products of heroin extracted in MeOH-DCM at 354°C

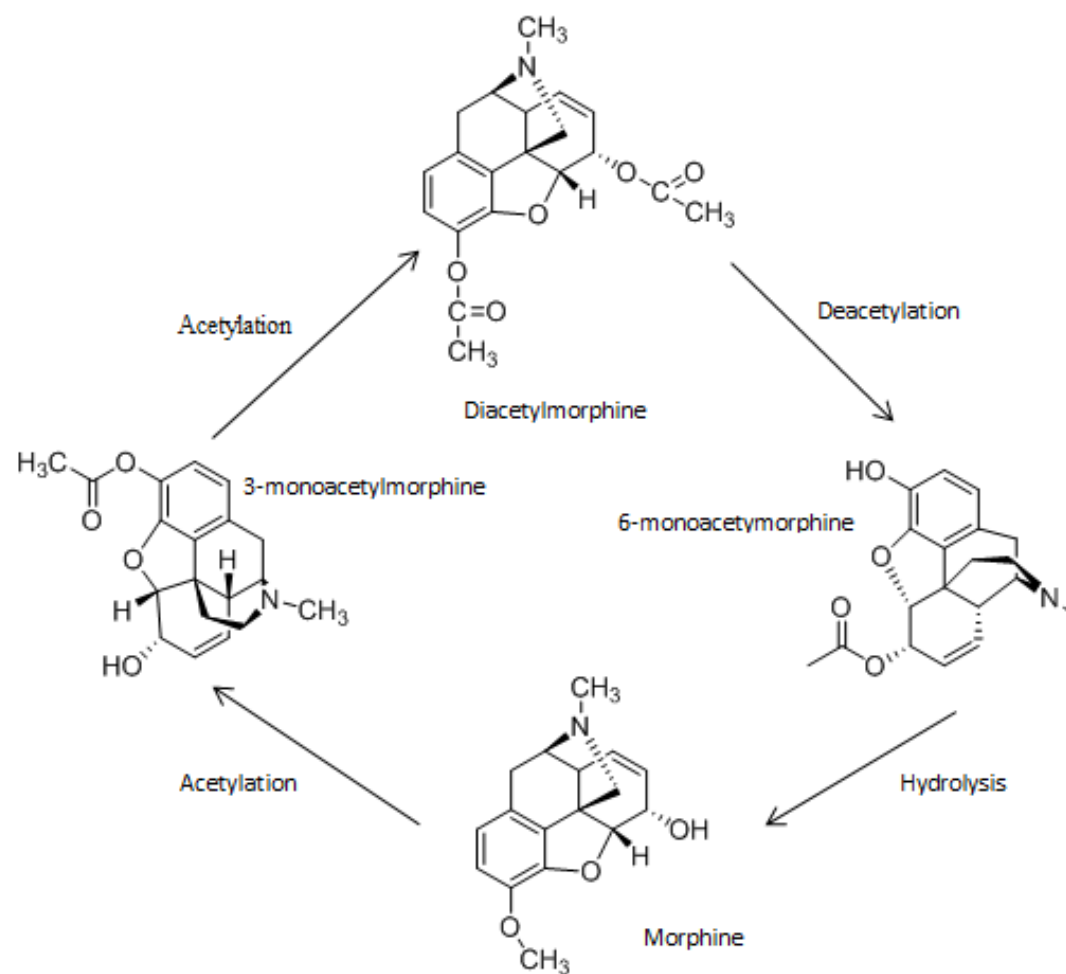


Figure 5.3 Chemical transformations of diacetylmorphine [186]



### **5.1.3 Method repeatability test for the pyrolysis products of heroin**

The repeatability of the method for the production of pyrolysis products of heroin was evaluated to determine the analytical repeatability for desorbed samples. This involved the establishment of repeatability of results within one sampling tin (within-can repeatability) and between the 6 six tin cans. For within-can repeatability, two sets of 6 replicate samples each were prepared by weighing 0.5g of heroin into 6 tin cans per set, these were heated and pyrolysis products trapped and extracted as per the protocol in 4.2.2.1. Each sampling tin can contained a total of 6 ACS strips. The trapped pyrolysis products from each set were desorbed using the appropriate solvent and ratio. Each resultant extract was analysed six times to facilitate the calculation of repeatability within a desorption and analysis event. Chromatographic profiles of pyrolysis products generated from each of the six replicate injections in each sample tin can were plotted and compared for similarities in retention time, peak shape, peak height as well as any differences in overall chemical profile.

Within-can repeatability was evaluated through %RSDs values calculated from average peak areas of pyrolysis products detected from the 6 replicate injections performed on each of the 6 strips in each tin can using both solvents and ratios.

To evaluate the in-between can repeatability, %RSD results for within-can repeatability for each pyrolysis product obtained from the 6 cans were compiled (average peak areas of pyrolysis products detected from the 6 injections in each of the 6 tin cans (n=36); their %RSDs values were calculated and used to establish between-can repeatability.

### **5.1.4 Repeatability test results**

Typical chromatographic profiles of the detected pyrolysis products obtained from the 6 injections in tin can 1, (extracted using both MeOH-DCM 90:10 and MeOH-DCM 50:50 solvents at 354<sup>0</sup>C) are displayed in figures 5.4 and 5.5 (appendix A). They all show similar retention times, similar pyrolysis products and quantities and a large paracetamol peak at 11.022 minutes. Tables 5.2 and 5.3 (appendix B) show

calculated %RSDs for tin can 1 using MeOH-DCM 90:10 and MeOH-DCM 50:50 respectively. Obtained %RSD values for the both solvents were in the analytical range of 0.01-2.72, indicating excellent repeatability.

Chromatographic profiles of pyrolysis products generated across the six different tin cans were also compared. This was followed by the calculation of %RSDs across these samples. Figures 5.6 and 5.7 (appendix A) shows chromatographic profiles of heroin pyrolysis products generated at 354<sup>0</sup>C for the MeOH-DCM 50:50 and 90:10 solvents respectively. Profiles revealed similar retention times but calculated %RSD values (tables 5.4 and 5.5) (appendix B) were all ≥5%, indicating that repeatability was considerably more challenging. This is not unreasonable given the nature of the pyrolysis event and that some variation would be expected to occur

Both chromatographic profiles revealed a large paracetamol peak at 11.02 minutes. A tentative explanation for the existence of this peak was that the paracetamol might have undergone some further decomposition in the GCMS where temperatures (injection port 280<sup>0</sup>C) were much higher than its melting point of 169<sup>0</sup>c.

### **5.1.5 Conclusion of results for the pyrolysis of heroin with the GCMS**

The results obtained from the analysis of pyrolysis products of heroin from a mixture of the two drugs revealed that repeatability is easily achieved for multiple single injections from ACS strips within one tin can and within one strip, indicating overall stability of the pyrolysis to analysis method. As expected the repeatability of analysis between sample cans varies much more widely. This is undoubtable as a result of the more unpredictability of the generation of pyrolysis products and is to be expected. One very useful result obtained, however is that the nature of the pyrolysis mixtures did not vary significantly within a specific temperature solvent ratio mix and this provides a considerable confidence to the methodology.

## **5.2 Isolation of pyrolysis products of EFV and heroin and analysis with the GCMS.**

### **5.2.1 Sample preparation**

Six replicate samples of EFV and heroin (from forensic case samples), were prepared by weighing 0.5g of each drug, mixing the two drugs together and then placing the mixture with 6 separate sampling tin cans. The mixture was heated to 354<sup>0C</sup> and pyrolysis products extracted according to the protocol described previously in section 4.2.2.1. The temperature was chosen as it provided the most quantity of the EFV pyrolysis product across both solvent ratios. The EFV was observed to undergo decomposition if heated above this temperature. This was described in detail in section 3.6.5.2. As previously described the pyrolysis products were trapped using four ACS in each of the six sampling cans where one of the four ACS strips was extracted with MeOH-DCM 90:10 and the other with MeOH-DCM 50:50 with the final two being reserved for HPLC analysis.

### **5.2.2 Results and Discussions**

Results obtained (table 5.6) revealed the presence of diacetylmorphine, 6-monoacetylmorphine, acetylcodeine, EFV, EFV product 1, hydrocortanine, caffeine, paracetamol and meconin lactone. Typical chromatograms of pyrolysis products obtained from the pyrolysis of heroin at 354<sup>0C</sup> (extracted using MeOH-DCM 90:10

and MeOH-DCM 50:50) are displayed in figure 5.10 and 5.11 respectively. The presence of some of these pyrolysis products in heroin samples (except EFV and EFV pyrolysis product 1) was discussed in detail earlier in this chapter, section 5.1.2, where they were reported as either alkaloids of the opium plant or breakdown products of these alkaloids. EFV was observed to undergo minimal decomposition when heated with heroin.

**Table 5.6 Pyrolysis products of EFV-heroin extracted in the two MeOH-DCM solvent ratios solvents at 354°C**

Pyrolysis Temperature (in °C)	GCMS EFV-heroin ACS	
	DCM:MeOH 50:50	DCM:MeOH 10:90
315	acetaminophen Meconin caffeine Morphine Acetyl codeine 6 Mono acetyl morphine Papaverine Noscapine EFV	acetaminophen Meconin Diacetamate Morphine Acetyl codeine 6 Mono acetyl morphine Papaverine EFV
354	acetaminophen Meconin lactone caffeine hydrocatamine morphine Acetyl codeine 6-monoacetyl morphine Papaverine EFV	acetaminophen Meconin lactone diacetamate caffeine hydrocatamine Acetyl codeine 6-monoacetyl morphine Diacetylmorphine EFV
394	acetaminophen Meconin lactone caffeine hydrocatamine EFV Acetyl codeine 6-monoacetyl morphine diacetylmorphine	acetaminophen Meconin lactone caffeine hydrocatamine EFV Acetyl codeine 6-monoacetyl morphine diacetylmorphine

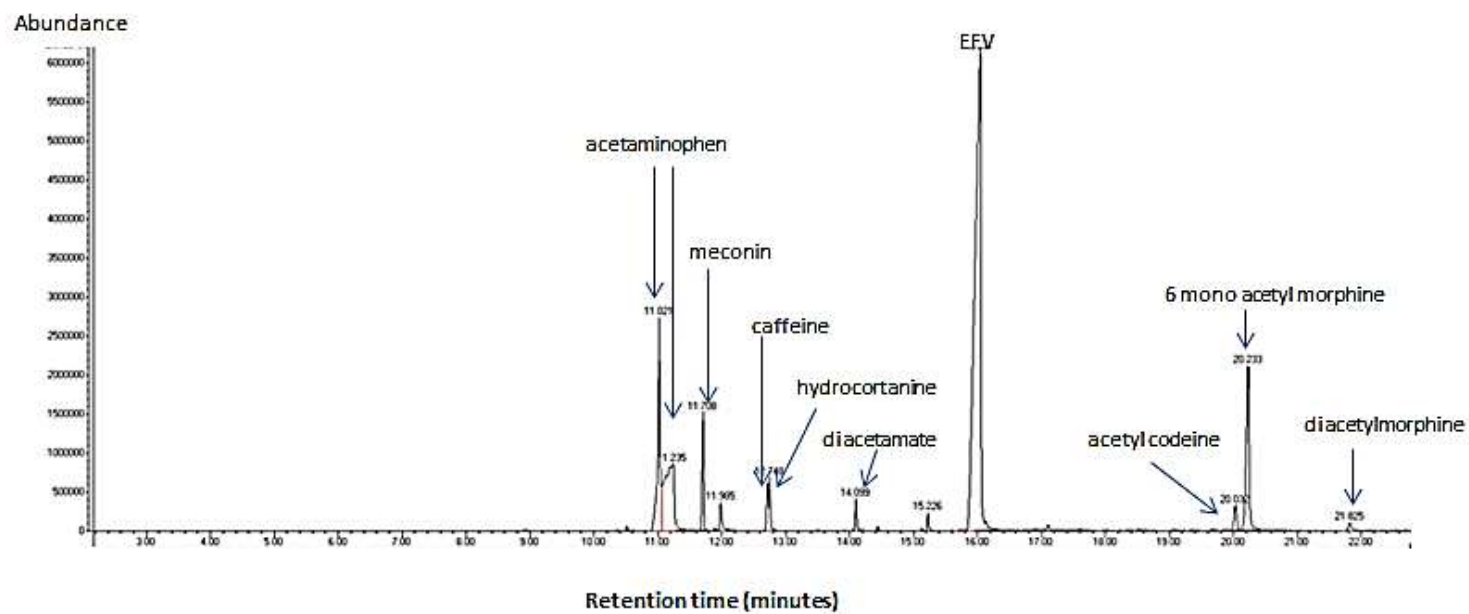


Figure 5.10 Chromatogram for pyrolysis products of heroin extracted in MeOH-DCM 50:50 at 354°C

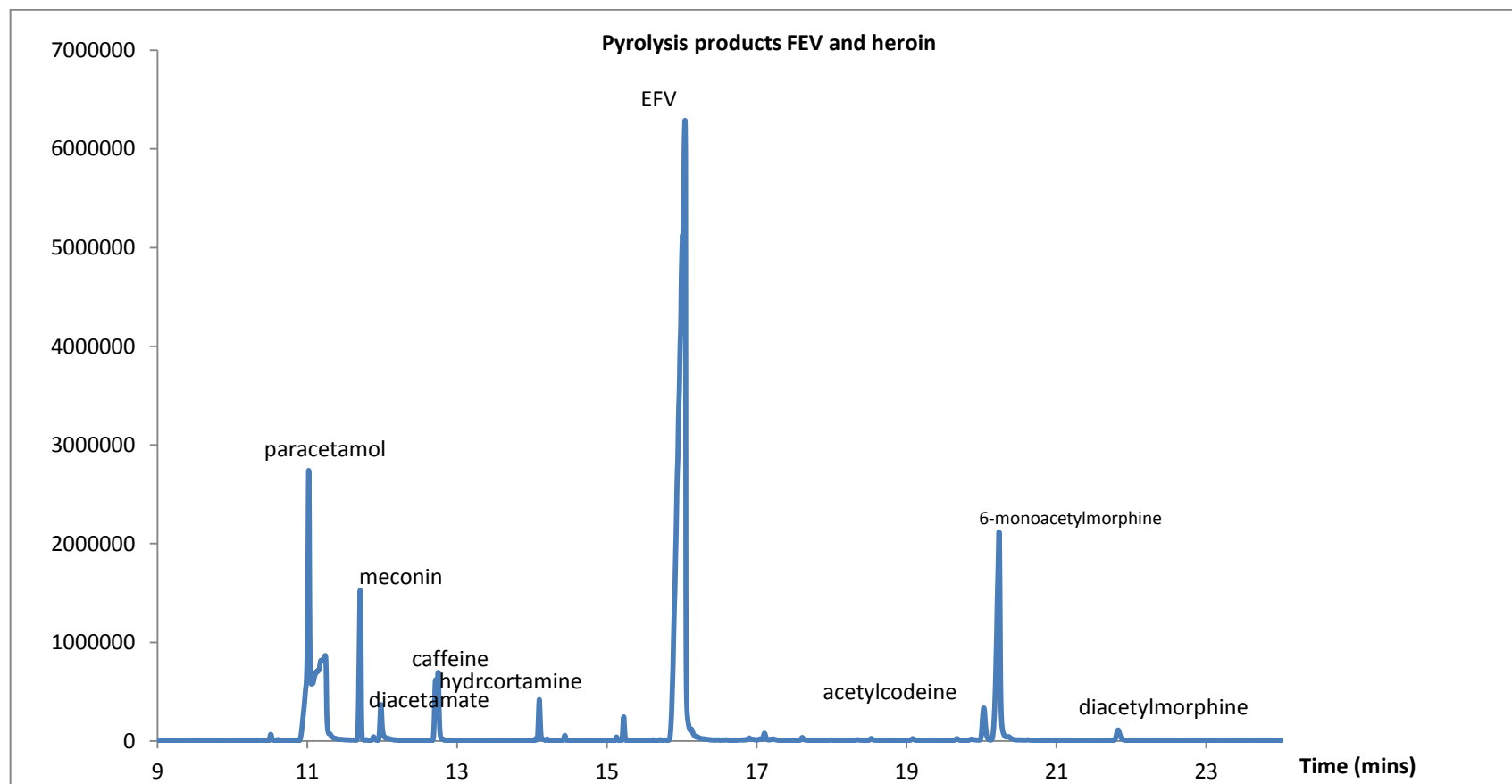


Figure 5.11 Pyrolysis products of EFV-heroin extracted in MeOH-DCM at 354°C

### **5.2.3 Repeatability test for the pyrolysis products of EFV and heroin**

The repeatability of the method for the production of pyrolysis products of EFV and heroin was evaluated to determine the analytical repeatability for desorbed samples. This involved the establishment of repeatability of results within one sampling tin (within-can repeatability) and between the 6 six tin cans (in-between can repeatability). For within-can repeatability, two sets of 6 replicate samples each were prepared by weighing 0.5g of methamphetamine into 6 tin cans per set, these were heated and pyrolysis products trapped and extracted as per the protocol in 4.2.2.1. Each sampling tin can contained a total of 6 ACS strips. The trapped pyrolysis products from each set were desorbed using the appropriate solvent and ratio. Each resultant extract was analysed six times to facilitate the calculation of repeatability within a desorption and analysis event. Chromatographic profiles of pyrolysis products generated from each of the six replicate injections in each sample tin can were plotted and compared for similarities in retention time, peak shape, peak height as well as any differences in overall chemical profile.

Within-can repeatability was evaluated through %RSDs values calculated from average peak areas of pyrolysis products detected from the 6 replicate injections performed on each of the 6 strips in each tin can using both solvents and ratios.

To evaluate the in-between can repeatability, %RSD results for within-can repeatability for each pyrolysis product obtained from the 6 cans were compiled (average peak areas of pyrolysis products detected from the 6 injections in each of the 6 tin cans (n=36); their %RSDs values were calculated and used to establish between-can repeatability.

#### **5.2.3.1 Repeatability test results for pyrolysis products of EFV and heroin**

Typical chromatographic profiles of the detected pyrolysis products from the 6 injections in tin can 1, (extracted using both MeOH-DCM 90:10 and MeOH-DCM 50:50 solvents at 354<sup>0</sup>C) are displayed in figures 5.12 and 5.13 (appendix A). They all show similar retention times, similar pyrolysis products and quantities and a large paracetamol peak at 11.022 minutes. Tables 5.2 and 5.3 (appendix B) show

calculated %RSDs for tin can 1 using MeOH-DCM 90:10 and MeOH-DCM 50:50 respectively. Obtained %RSD values for both solvents were in the analytical range of 0.12-5.44, indicating excellent repeatability.

Chromatographic profiles of pyrolysis products generated across the six different tin cans were also compared. This was followed by the calculation of %RSDs across these samples. Figures 5.14 and 5.15 (appendix A) shows chromatographic profiles of heroin pyrolysis products generated at 354<sup>0</sup>C for the MeOH-DCM 50:50 and 90:10 solvents respectively. Profiles revealed similar retention times but calculated %RSD values (tables 5.4 and 5.5) (appendix B) were all ≥5%, indicating that repeatability was considerably more challenging. Plots of pyrolysis product versus product peak area (figure 5.16 and 5.17) for heroin pyrolysis products also revealed variations in product quantities. This is not unreasonable given the nature of the pyrolysis event and that some variation would be expected.

Both chromatographic profiles revealed a large paracetamol peak at 11.02minutes; a tentative explanation for the existence of this peak was that the paracetamol might have undergone decomposition in the GCMS where temperatures (injection port 280<sup>0</sup>C) were much higher than its melting point of 169<sup>0</sup>c.

The results obtained from the analysis of pyrolysis products of heroin from a mixture of the two drugs also revealed that repeatability is easily achieved for multiple single injections from the 6 ACS strips within a tin can and within one strip, indicating overall stability of the pyrolysis to analysis method. As expected the repeatability of analysis between sample cans varies much more widely. This is undoubtable as a result of the more unpredictability of the generation of pyrolysis products and is to be expected. One very useful result obtained, however is that the nature of the pyrolysis mixtures did not vary significantly within a specific temperature solvent ratio mix and this provides a considerable confidence to the methodology.



It was observed that the presence of EFV in the mixture did not interfere with the pyrolysis of heroin; however the EFV was observed to have remained stable and did not undergo any transformation.

### **5.3 Isolation of pyrolysis products of heroin and analysis with the HPLC**

Heroin samples used for the HPLC analysis were the two remaining ACS strips from the four prepared and detailed in section 5.1.1. In this case two of the ACS strips were analysed using the HPLC where one was extracted using MeOH-DCM 90:10 and the second extracted using the mobile phase. The samples were obtained from finished forensic work.

#### **5.3.1 Results and discussions**

The results obtained (table 5.11 and figure 5.16a) for extractions with the mobile phase revealed the presence of morphine, meconin, noscapine, papaverine and other unidentified products, while those extracted with MeOH-DCM 90:10 v/v (table 5.12 and figure 5.16b) showed the presence of meconin, noscapine and papaverine and other some unidentified products. All detected products were alkaloids of opium; their presence was expected since these are street samples where purity is not always prioritised. Cutting agents and other impurities have been reported in street samples [24- 29]. The presence of morphine amongst the pyrolysis products of heroin is indicative of incomplete deacetylation reaction.

**Table 5.11 Pyrolysis of heroin extracted in the mobile phase at 354<sup>0</sup>C**

Pyrolysis Temperature ( in degrees Celsius)	HPLC heroin	
	Mobile phase 25Mm buffer-ACN (53:47)	Retention time (minutes)
315	Morphine	1.35
	Meconin	1.72
	Noscapine	2.85
	papaverine	8.45
354	Morphine	1.35
	Meconin	1.72
	Noscapine	2.85
	papaverine	8.45
358	Morphine	1.35
	Meconin	1.72
	Noscapine	2.85
	papaverine	8.45

**Table 5.12 Pyrolysis products of heroin extracted in MeOH:DCM 90:10 at 354<sup>0</sup>C**

Pyrolysis Temperature ( in degrees Celsius)	HPLC heroin	
	MeOH-DCM 9010 v/v	Retention time (minutes)
315	Meconin	1.91
	Noscapine	2.75
	papaverine	7.90
354	Meconin	1.91
	Noscapine	2.75
	papaverine	7.90
358	Meconin	1.91
	Noscapine	2.75
	papaverine	7.90

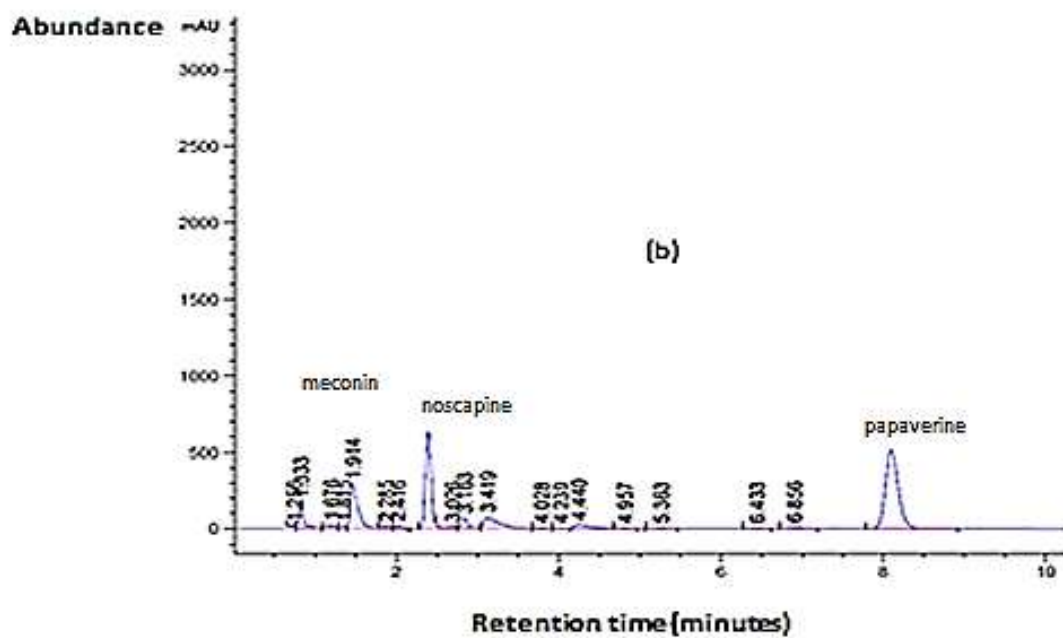
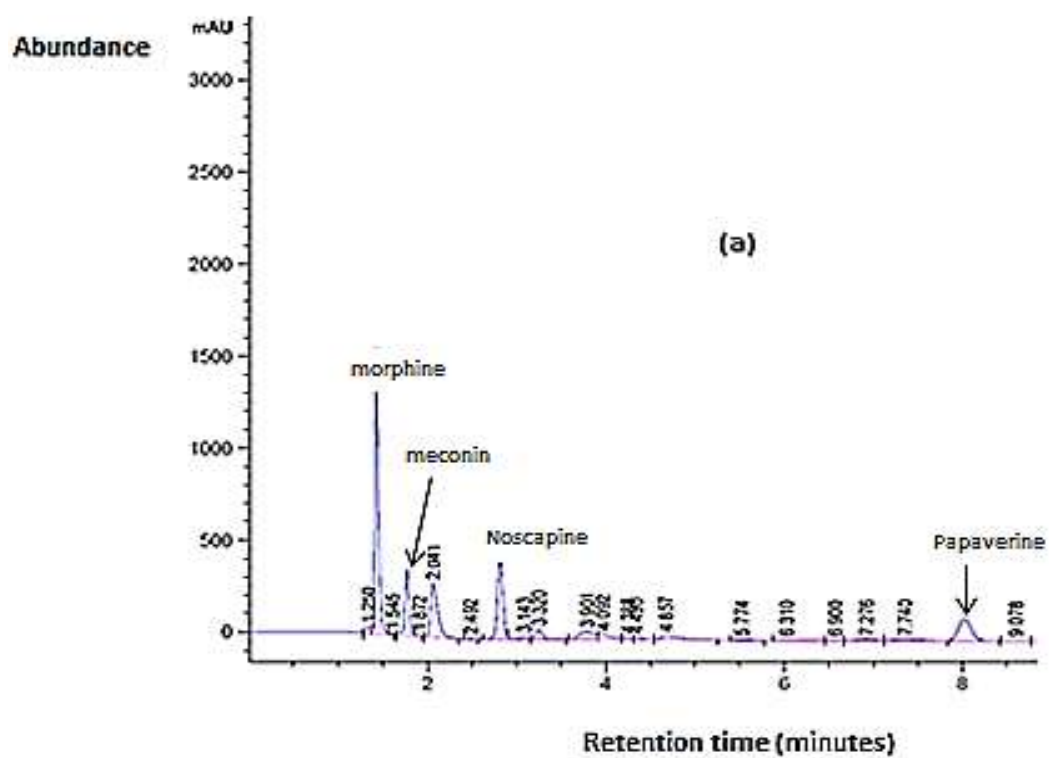


Figure 5.16 Chromatograms of pyrolysis products of heroin extracted in (a) the mobile phase and (b) MeOH-DCM 90:10 at 354°C

### 5.3.2 Method repeatability test for pyrolysis products of heroin

The repeatability of the analytical methods (pyrolysis generation and desorption) method for heroin pyrolysis products using the HPLC was evaluated through calculation of %RSDs from peak areas. Average peak areas for all the 6 replicate injections from the 6 ACS in sample can 1 were used to represent the within-can analytical repeatability. The results obtained for samples pyrolysed at 354<sup>o</sup>C from the mobile phase are presented in table 5.13 while those from the MeOH-DCM (90:10) are in table 5.14 (appendix B) Both results revealed %RSD values of  $\leq 5\%$ , indicating very good repeatability.

The repeatability of inter-can results was also evaluated. This was obtained from %RSDs values calculated from average peak areas of pyrolysis products generated from the 6 injections in each of the 6 sample cans (n=36). The results obtained using the mobile phase are presented in table 5.15 while those for the MeOH:DCM 90:10 are in table 5.16 (appendix B). Both results revealed %RSD values of  $\geq 5\%$ , indicating that repeatability was poorer as expected for this kind of work. Plots of peak area versus pyrolysis (figures 5.17 and 5.18) (appendix A) also revealed variations in the quantities of pyrolysis products.

#### **5.4 Isolation of pyrolysis products from a mixture of EFV and heroin and analysis with the HPLC**

Six replicate samples of EFV and heroin from finished case work were prepared by weighing 0.5g of each drug, mixing together and placing the mixture into a sample can. 6 mixed samples were prepared in this way. The mixture was heated to 354°C and pyrolysis products extracted according to the protocol described previously.

##### **5.4.1 Results and Discussions**

The results obtained (Table 5.17) (figure 5.19a) for mobile phase extractions and (Table 5.18 (figure 5.19b) for the MeOH-DCM 90:10 v/v revealed the presence of the same set of pyrolysis products in both extracts. This consisted of a mixture of meconin, acetyl codeine, 3.6 acetyl morphine, noscapine, EFV and EFV pyrolysis product 1. The first three compounds are breakdown products from alkaloids of opium and are expected due to the type of samples analysed.

**Table 5.17 Pyrolysis products of EFV-heroin extracted in the mobile phase**

Pyrolysis Temperature ( in degrees Celsius)	HPLC EFV and heroin	
	Mobile phase 25Mm buffer-ACN (53:47)	Retention time (minutes)
315	EFV product 1	1.01
	meconin	1.70
	unidentified peak	2.11
	codeine	2.80
	EFV	10.07
354	EFV product 1	1.01
	meconin	1.70
	unidentified peak	2.11
	codeine	2.80
	EFV	10.07

**Table 5.18 Pyrolysis products of EFV-heroin extracted in MeOH-DCM 90:10**

Pyrolysis Temperature ( in degrees Celsius)	HPLC EFV and heroin	
	Mobile phase 25Mm buffer-ACN (53:47)	Retention time (minutes)
315	EFV product 1	1.12
	Meconin	1.70
	Noscapine	2.79
	Acetylcodeine	3.88
	3,6 acetyl morphine	4.80
	EFV	10.09
354	EFV product 1	1.12
	Meconin	1.70
	Noscapine	2.79
	Acetylcodeine	3.88
	3,6 acetyl morphine	4.80
	EFV	10.09

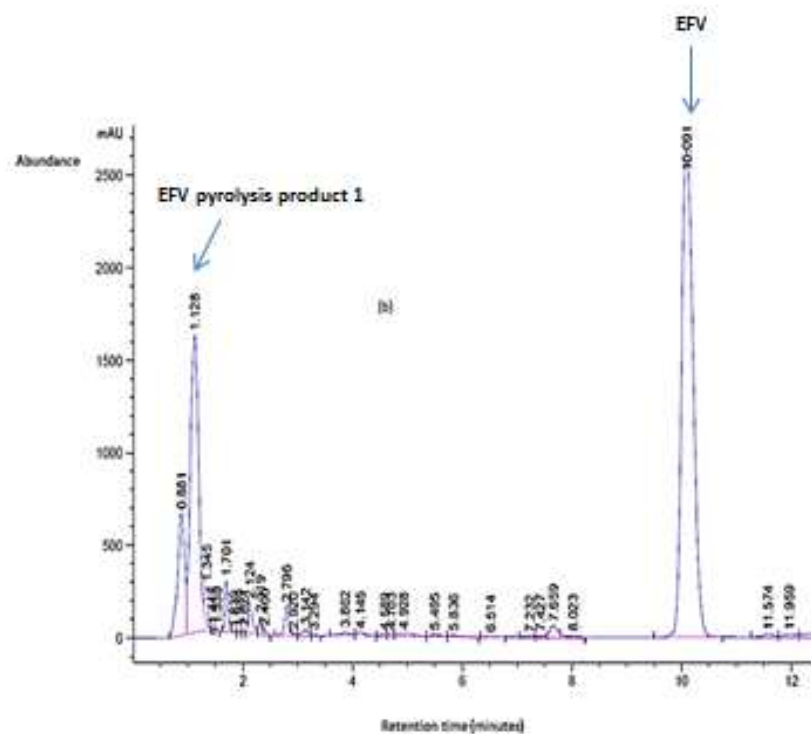
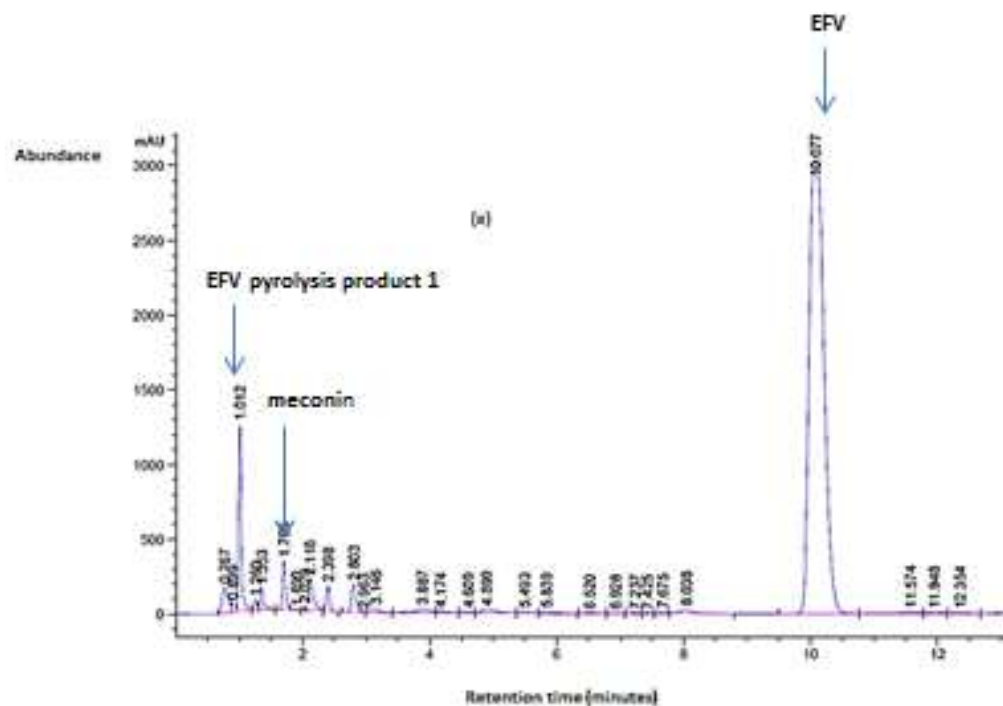
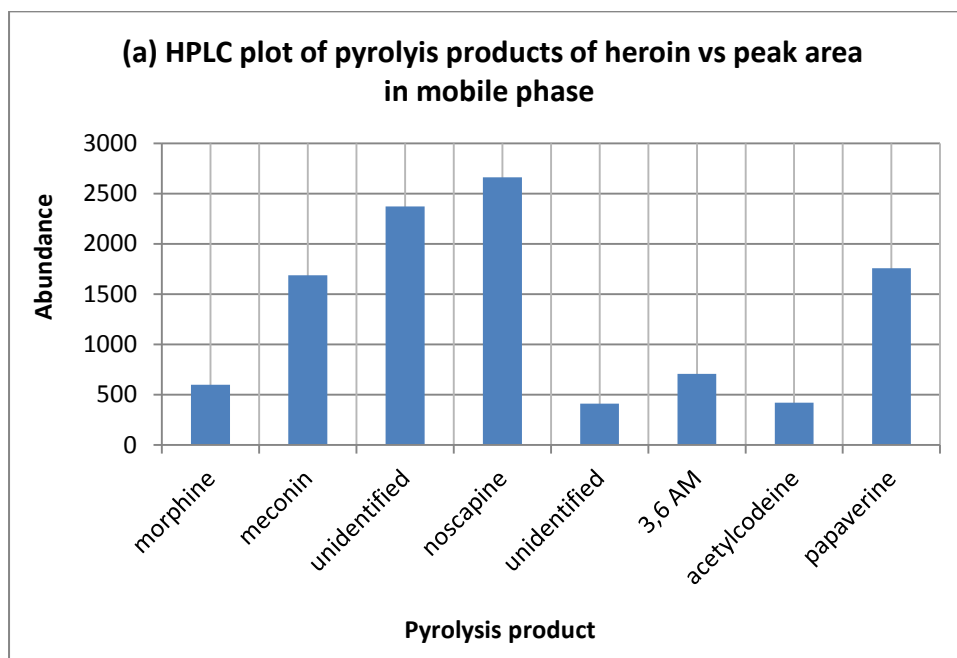


Figure 5.19 Chromatograms of pyrolysis products of EFV and heroin extracted using the a) mobile phase and b) MeOH-DCM 90:10 (b)

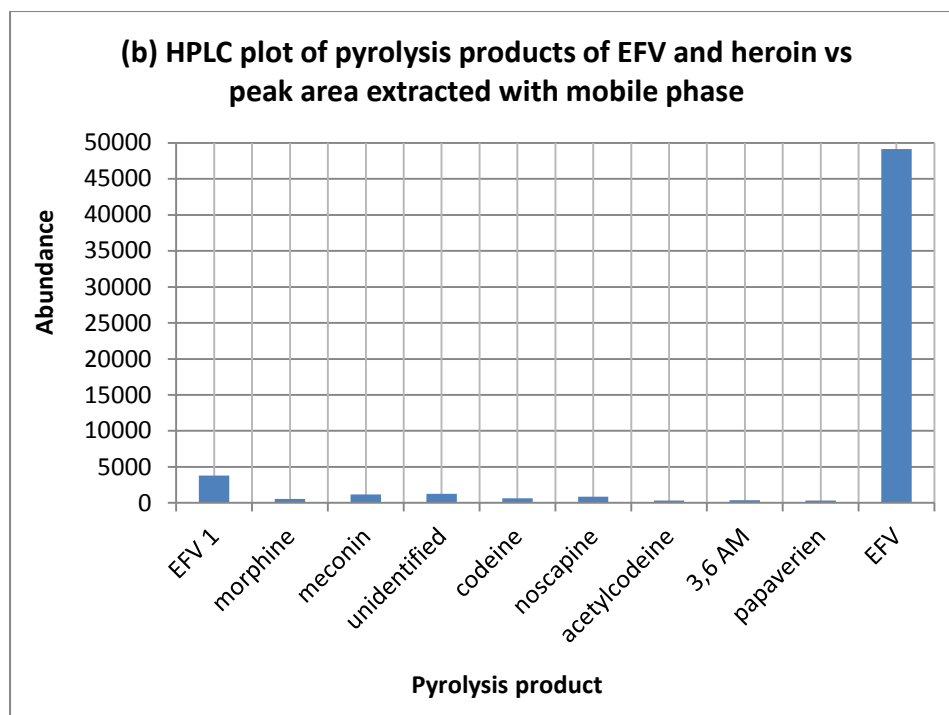
#### 5.4.2 Method repeatability test for pyrolysis products of EFV-heroin

The repeatability of the method for the EFV-heroin pyrolysis products was evaluated through the calculation of %RSDs of product peak areas extracted using both solvents. The peak areas for all the 6 replicates injections from the 6 ACS in one sample can as before provided an extraction to instrument analytical check (tables 5.19 and 5.20) (appendix B) indicating very good repeatability. The inter-can analytical repeatability presented in tables 5.21 and 5.22 (appendix B) were more variable.

Plots of peak area versus pyrolysis (figures 5.20 and 5.21) (appendix A) also revealed variations in the quantities of detected pyrolysis products. Quantities of the pyrolysis products for when heroin was heated on its own (figure 4. 28a) were compared with those obtained from when heating its mixture with EFV; figure 4.27 shows results obtained from extractions with the mobile phase. The two results were plotted against each other for a better comparison.







**Figure 5.22 Comparison of quantities of pyrolysis products of heroin after it was a) heated alone (a) and (b) when it was heated in EFV – both extracted with the mobile phase**

The results showed that more pyrolysis products were obtained when heroin was heated on its own. They also showed that the EFV underwent very little decomposition when heated with heroin. Based on these findings, it can be concluded that when a mixture of both drugs is smoked for abuse, the inhaled pyrolysis products are likely to contain varying amounts of a mixture of products from both drugs and that the products are likely to contain more EFV compound than others. These finding correlate with those obtained from the GCMS.

### **5.5 Summary of the pyrolysis EFV and heroin.**

The pyrolysis of heroin revealed pyrolysis product formation via processes of deacetylation and acetylation. Other detected pyrolysis products were cutting agents like caffeine and paracetamol, which are commonly found in heroin street samples. Just like with the ATS, the presence of EFV in a mixture of heroin did not alter the expected pyrolysis reactions of heroin. However EFV was found to

undergo very little decomposition when heated with heroin. A conclusion drawn from the pyrolysis of the two drugs was that when the two are smoked together as a mixture, the inhaled pyrolysis products will likely comprise of a higher quantity of the EFV compound than those of heroin.

## CHAPTER 6: EFFECTS OF EFAVIRENZ ON NATURAL DRUGS ALLEGED TO BE IN THE NYAOPE MIXTURE

### 6.1 Isolation of pyrolysis products of opium and analysis with the GCMS

#### 6.1.1 Sample preparation

Opium samples were first homogenised by crushing them into fine powder using a mortar and pestle. 0.5g of the powdered material was weighed into 6 separate tin cans. Each tin can was covered with a lid, heated to the desired temperature and removed from the hot plate. The covering lid was then replaced with the one with prepared ACS strips (section 4.2.2.1) and incubated for 16 hours at 80<sup>0</sup>C to trap the pyrolysis products. The products were trapped using four ACS in each tin can. Following this, the analysis sampling strategy in section 4.2.2.2 was applied. For GCMS analysis one of the four ACS strips was extracted with MeOH-DCM 90:10 and the other with MeOH-DCM 50:50. The samples were obtained from finished forensic cases.

#### 6.1.2 Results and Discussions

Results obtained from samples extracted with the two different solvents ratios are displayed in table 6.1. They showed the detection of noscapine, papaverine, thebaine, codeine, morphine, hydrocortanine and *6,7-dimethoxy-1(3H)-Isobenzofuranone* (meconin). The first five compounds are the main alkaloids of opium while hydrocortanine and *6,7-dimethoxy-1(3H)-Isobenzofuranone*, are breakdown products of noscapine [191]. Noscapine is the second most abundant alkaloid in opium poppy latex after morphine, with the highest content found in Russian and Indian opium at 12 and 7.7%, respectively [191-193]. Typical chromatograms for pyrolysis products obtained from the pyrolysis of opium at 354<sup>0</sup>C (extracted using MeOH-DCM 90:10 and 50:50) are displayed in figure 6.1 and 6.2 respectively.

**Table 6.1 Pyrolysis products of opium extracted in the two solvents at various temperatures**

ACS extracting solvent	Pyrolysis Temperature (°C)		
	315	354	396
<b>MeOH:DCM 50:50</b>	Meconin Hydrocortanine Codeine Morphine thebaine papaverine noscapine	Meconin Hydrocortanine Codeine Morphine thebaine papaverine noscapine	Meconin Hydrocortanine Codeine Morphine thebaine papaverine noscapine
<b>MeOH:DCM 90:10</b>	Meconin Hydrocortanine Codeine Morphine Thebaine papaverine	Meconin Hydrocortanine Codeine Morphine Thebaine papaverine	Meconin Hydrocortanine Codeine Morphine Thebaine papaverine

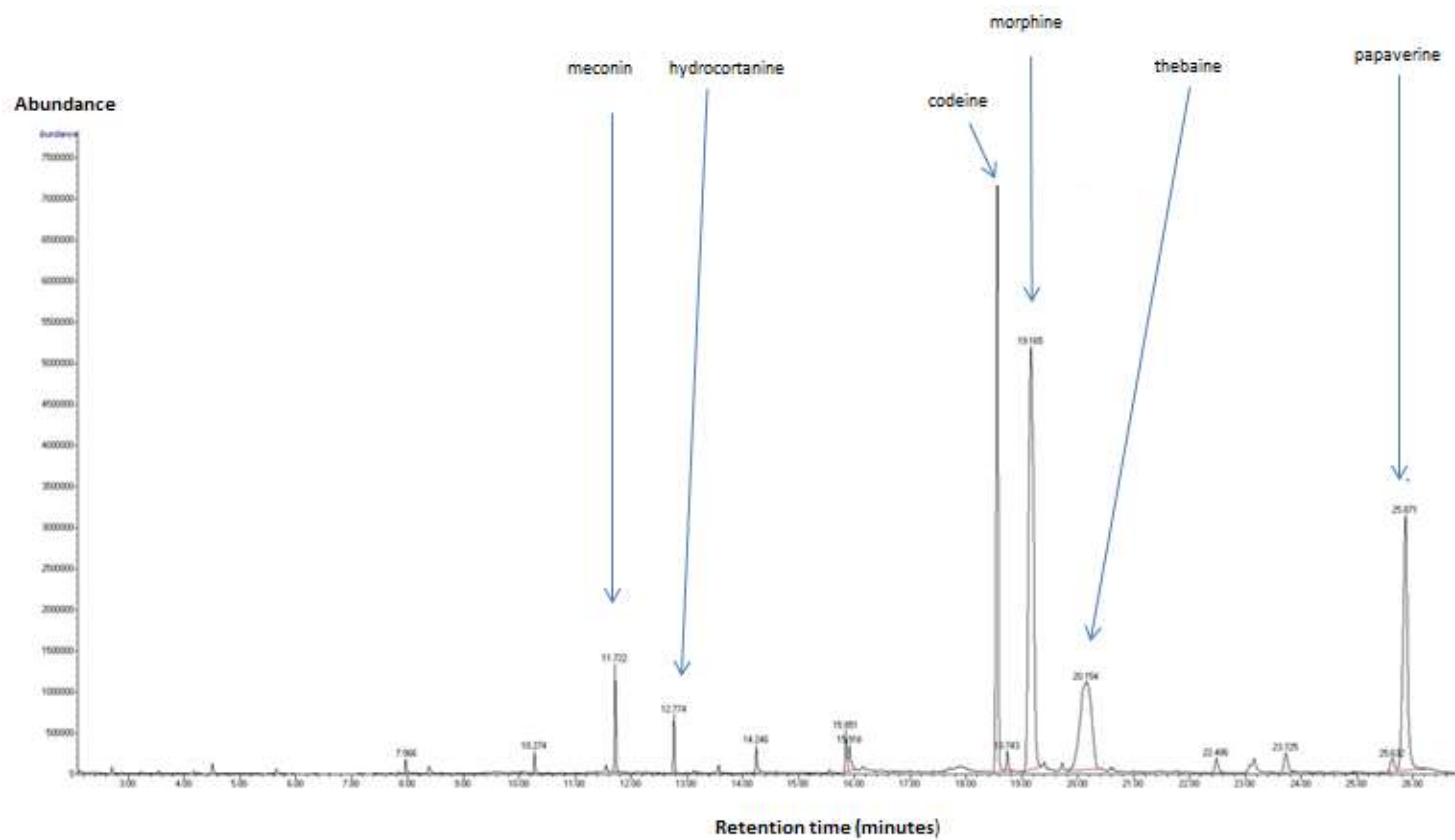


Figure 6.1 Chromatogram for pyrolysis products of opium extracted in MeOH-DCM 90:10 at 354<sup>0</sup>C

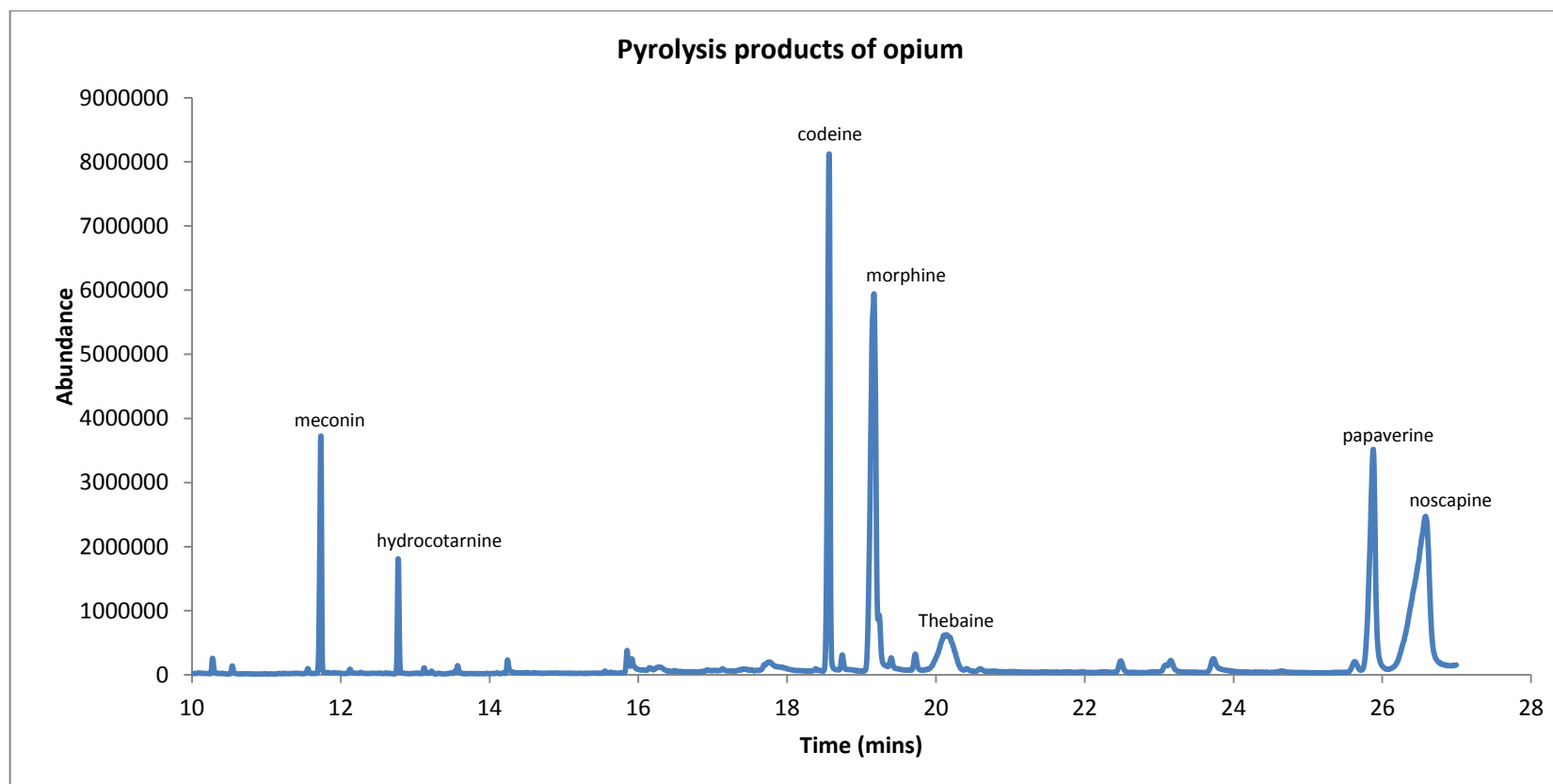


Figure 6.2 Chromatogram for pyrolysis products of opium extracted in MeOH-DCM 50:50 at 354<sup>0</sup>C

### **6.1.3 The repeatability of the method for the production of pyrolysis products of opium**

The repeatability of the method for the production of pyrolysis products of heroin was evaluated to determine the analytical repeatability for desorbed samples. This involved the establishment of repeatability of results within one sampling tin (within-can repeatability) and between the 6 six tin cans. For within-can repeatability, two sets of 6 replicate samples each were prepared by weighing 0.5g of methamphetamine into 6 tin cans per set, these were heated and pyrolysis products trapped and extracted as per the protocol in 4.2.2.1. Each sampling tin can contained a total of 6 ACS strips. The trapped pyrolysis products from each set were desorbed using the appropriate solvent and ratio. Each resultant extract was analysed six times to facilitate the calculation of repeatability within a desorption and analysis event. Chromatographic profiles of pyrolysis products generated from each of the six replicate injections in each sample tin can were plotted and compared for similarities in retention time, peak shape, peak height as well as any differences in overall chemical profile.

Within-can repeatability was evaluated through %RSD values calculated from average peak areas of pyrolysis products detected from the 6 replicate injections performed on each of the 6 strips in each tin can using both solvents and ratios.

%RSD results for within-can repeatability for each pyrolysis product obtained from the 6 cans were compiled (average peak areas of pyrolysis products detected from the 6 injections in each of the 6 tin cans (n=36); their %RSDs values were calculated and used to establish between-can repeatability.

#### **6.3.1.1 Repeatability test results**

Typical chromatographic profiles of the detected pyrolysis products obtained from the 6 injections in tin can 1, (extracted using both MeOH-DCM 90:10 and MeOH-DCM 50:50 solvents at 354<sup>0</sup>C) are displayed in figures 6.3 and 6.4. They all show similar retention times, similar pyrolysis products and quantities. Tables 6.2 and 6.3 show calculated %RSDs for tin can 1 using MeOH=DCM 90:10 and MeOH-DCM

50:50 respectively. Obtained %RSD values for the both solvents were in the analytical range of  $\leq 5\%$ , indicating excellent repeatability.

Chromatographic profiles of pyrolysis products generated across the six different tin cans were also compared. This was followed by the calculation of %RSDs across these samples. Figures 6.3 and 6.4 shows chromatographic profiles of heroin pyrolysis products generated at  $354^{\circ}\text{C}$  for the MeOH-DCM 50:50 and 90:10 solvents respectively. Profiles revealed similar retention times but calculated %RSD values (tables 6.4 and 6.5) were all  $\geq 5\%$ , indicating that repeatability was considerably more challenging. Plots of pyrolysis product versus product peak area (figure 6.5 and figure 6.6) for pyrolysis products of opium also revealed variations in product quantities. This is not unreasonable given the nature of the pyrolysis event and that some variation would be expected to occur.

## **6.2 Isolation of pyrolysis products of opium and analysis with the HPLC**

### **6.2.1 Sample preparation**

Opium samples used for the HPLC analysis were the two remaining ACS strips from the four prepared and detailed in section 5.1.1. In this case two of the ACS strips were analysed using the HPLC where one was extracted using MeOH-DCM 90:10 and the second extracted using the mobile phase. The samples were obtained from finished forensic work.

#### **6.2.1.1 Results and Discussions**

The results obtained (table 6.6 and figure 6.7a) and (table 6.7 and figure 6.7b) for extractions using the mobile phase and for the MeOH-DCM 90:10 respectively, revealed the detection of the same pyrolysis products of meconin, codeine, morphine, noscapine, and papaverine. The last four pyrolysis products were alkaloids of opium while meconin is a breakdown product of noscapine.

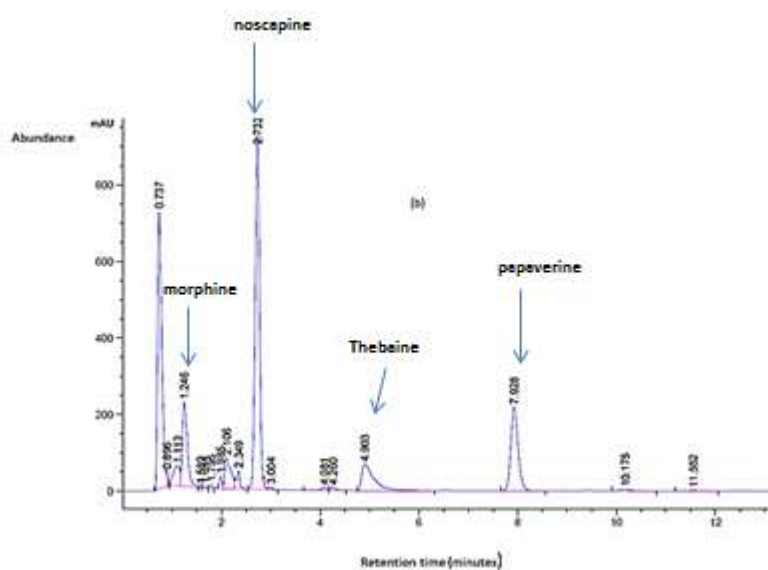
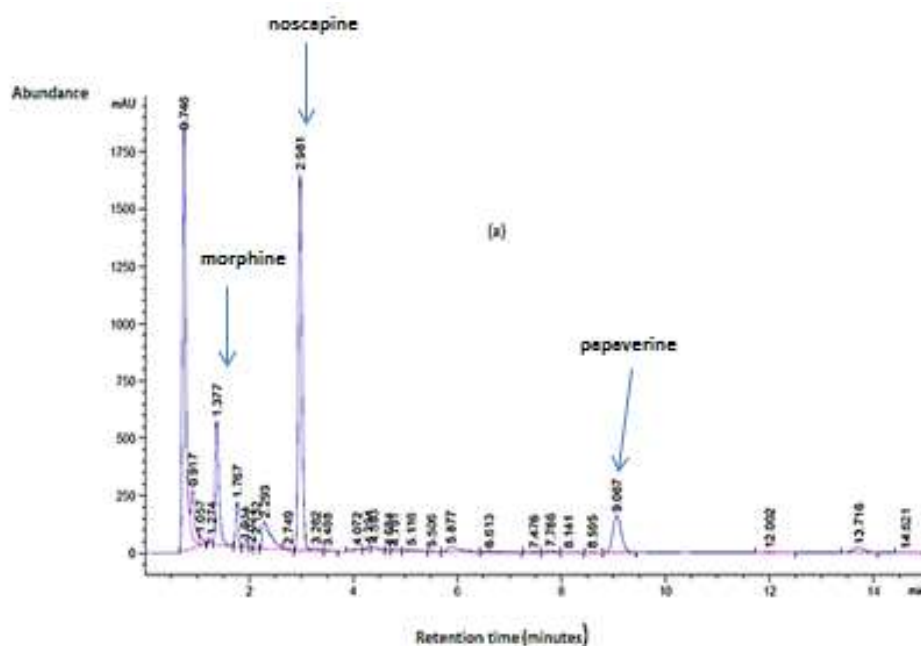


**Table 6.6 Results for pyrolysis products of opium extracted in the HPLC mobile phase**

Pyrolysis Temperature ( in degrees Celsius)	HPLC opium	
	Mobile phase 25Mm buffer-ACN (53:47)	Retention time (minutes)
315	Morphine	1.31
	Meconin	1.76
	Codeine	2.29
	Noscapine	2.98
	Thebaine	5.87
	papaverine	9.06
358	Morphine	1.31
	Meconin	1.76
	Codeine	2.29
	Noscapine	2.98
	Thebaine	5.87
	papaverine	9.06
398	Morphine	1.31
	Meconin	1.76
	Codeine	2.29
	Noscapine	2.98
	Thebaine	5.87
	papaverine	9.06

**Table 6.7 Results for the pyrolysis products of opium extracted in the MeOH-DCM 90:10**

Pyrolysis Temperature ( in degrees Celsius)	HPLC opium	
	Mobile phase 25Mm buffer-ACN (53:47)	Retention time (minutes)
315	Morphine Codeine Noscapine Thebaine papaverine	1.24 2.34 2.73 4.90 7.92
354	Morphine Codeine Noscapine Thebaine papaverine	1.24 2.34 2.73 4.90 7.92
358	Morphine Codeine Noscapine Thebaine papaverine	1.24 2.34 2.73 4.90 7.92



**Figure 6.7 Chromatogram of pyrolysis products of opium extracted using the mobile phase (a) and MeOH-DCM 90:10**

The results obtained revealed the detection of all five major alkaloids of opium and also the presence of the meconin, which is a breakdown product of noscapine.

Although noscapine is known to be the second most abundant opium alkaloid after morphine, it was detected in a rather large amount, which might suggest its use as a cutting agent in the analysed samples. The use of noscapine as a cutting agent in

heroin samples has been reported [191]. Noscapine is also a rather larger molecule (figure 5.3) (molecular weight 413.4 g/mol) when compared to other alkaloids of opium, and may not be easy to be broken down. Its analysis with the GCMS following ACS desorption might have helped with further decomposition and enabled its detection in larger amounts than on the HPLC.

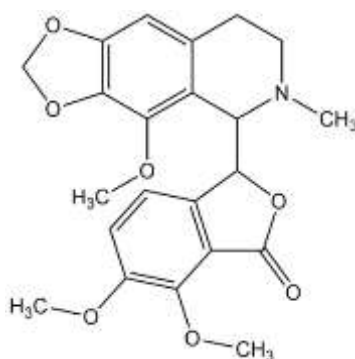


Figure 6.8 Chemical structure for noscapine [191]

### 6.3 Isolation of pyrolysis products of EFV and opium and analysis with the GCMS.

#### 6.3.1 Sample preparation

Six replicate samples of EFV and opium were prepared by weighing 0.5g of each drug, mixing the two drugs together and then placing the mixture with 6 separate sampling tin cans. The mixture was heated to 354<sup>0C</sup> and pyrolysis products extracted according to the protocol described previously in section 4.2.2.1. The temperature was chosen as it provided the most quantity of the EFV pyrolysis product across both solvent ratios. Work regarding the selection of suitable EFV pyrolysis temperature was discussed in detail in chapter 3.4.3. The pyrolysis products were trapped using four ACS in each of the six sampling cans where one of the four ACS strips was extracted with MeOH-DCM 90:10 and the other with MeOH-DCM 50:50 with the final two being reserved for HPLC analysis. Heroin samples were obtained from finished forensic work.

### 6.3.2 Results and Discussions

The results obtained from the pyrolysis of a mixture EFV and opium revealed the presence of meconin, EFV and its pyrolysis product 1 (table 6.8) and across the 6 tin cans using both solvent ratios. Typical chromatograms for pyrolysis products obtained from the pyrolysis of opium at 354<sup>0</sup>C (extracted using MeOH-DCM 50:50) is displayed in figure 6.9 and figure 6.10 (for the MeOH-DCM 90:10).

The EFV was found to produce the expected pyrolysis products when it heated with opium, but its presence in the mixture was discovered to mask the pyrolysis of opium. The presence of opium on the other hand did not affect the expected pyrolysis pattern for EFV.

**Table 6.8 Pyrolysis products of EFV and opium extracted in the two MeOH-DCM solvent ratios at 354<sup>0</sup>C**

ACS extracting solvent	Pyrolysis Temperature (°C)		
	315	354	396
<b>MeOH:DCM 50:50</b>	Meconin EFV EFV pyrolysis product 1	Meconin EFV EFV pyrolysis product 1	Meconin EFV EFV pyrolysis product 1
<b>MeOH:DCM 90:10</b>	Meconin EFV EFV pyrolysis product 1	Meconin EFV EFV pyrolysis product 1	Meconin EFV EFV pyrolysis product 1

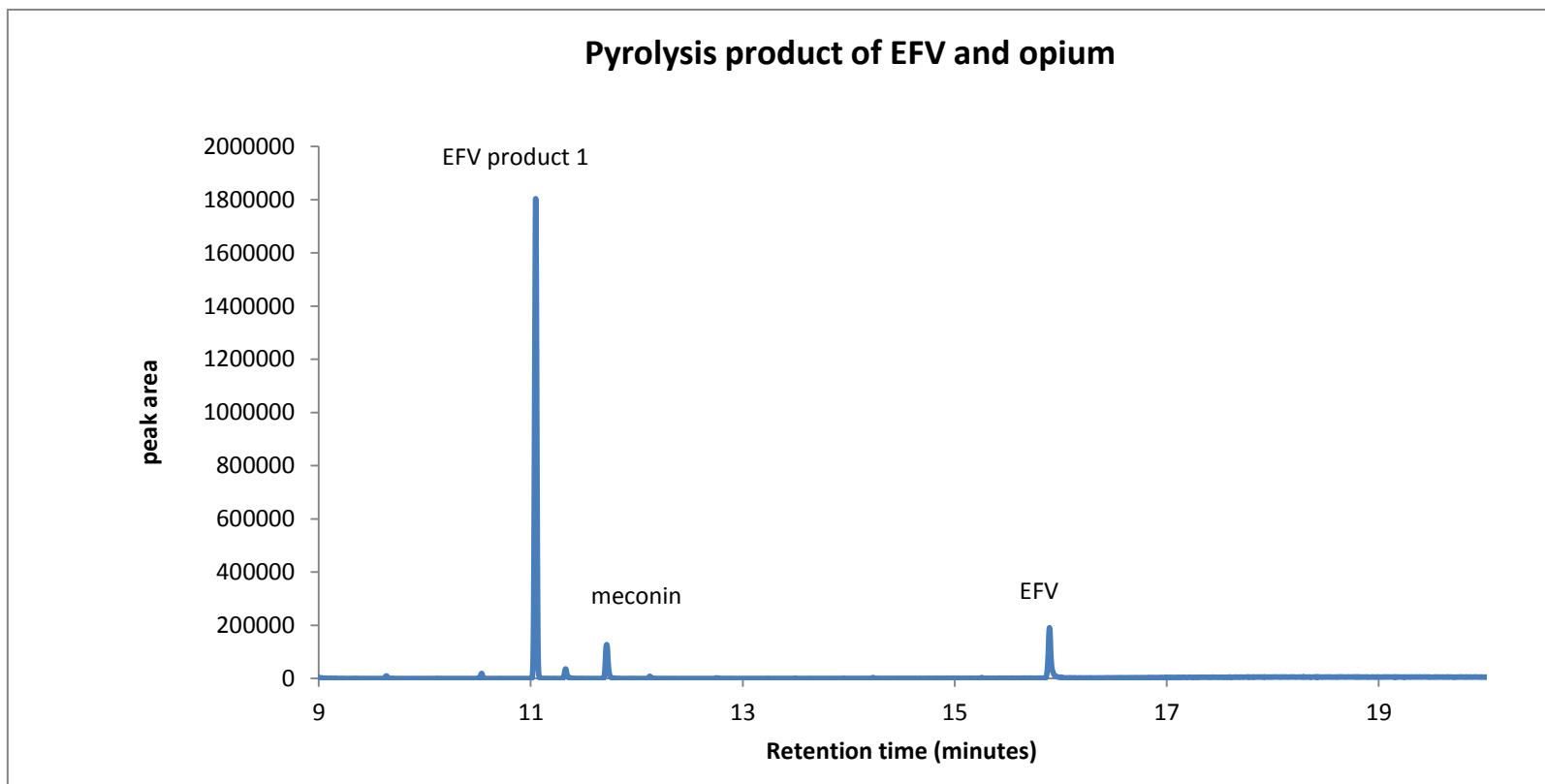


Figure 6.9 Chromatogram for pyrolysis products of EFV-opium extracted in MeOH-DCM 90:10 at 354<sup>0</sup>C

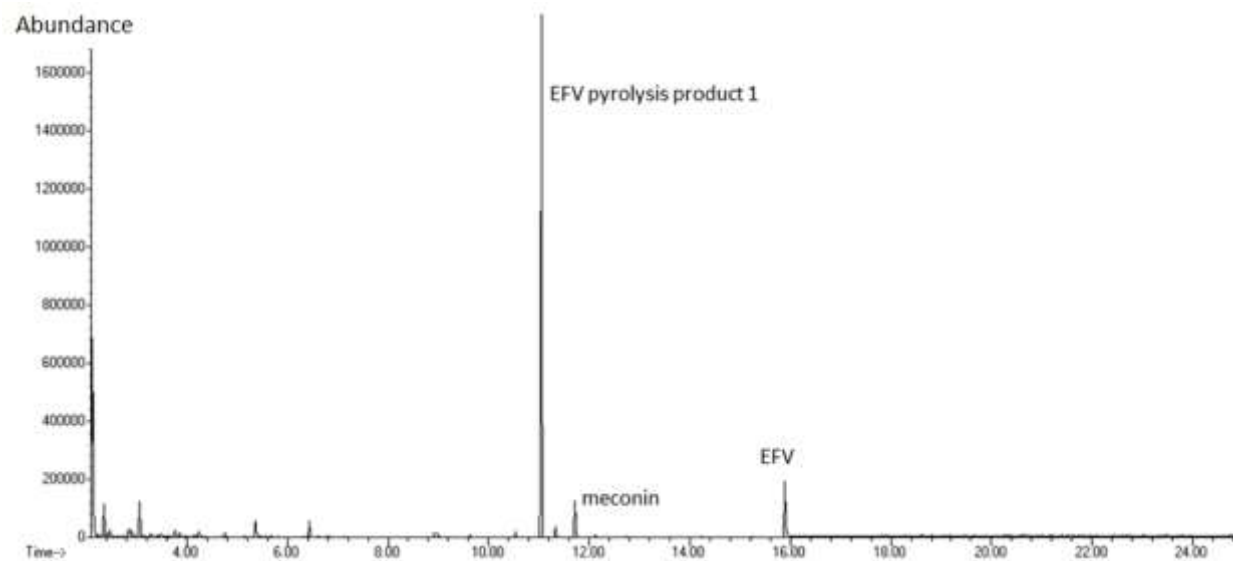


Figure 6.10 Chromatogram for the pyrolysis products of EFV-opium extracted in MeOH-DCM 50:50 at 354°C



The repeatability of the method for detected pyrolysis products of EFV and opium using the GCMS was not performed. This was because the repeatability of all the three detected products had already been performed for similar drug i.e. heroin, where within can repeatability was found to be excellent while the inter can results indicated that repeatability was not achievable for all the three pyrolysis products.

#### **6.4 Isolation of pyrolysis products of EFV and opium and analysis with the HPLC**

##### **6.4.1 Sample preparation**

Six replicate samples of EFV and opium from finished case work were prepared by weighing 0.5g of each drug, mixing together and placing the mixture into a sample can. 6 mixed samples were prepared in this way. The mixture was heated to 354<sup>0</sup>C and pyrolysis products extracted according to the protocol described previously.

##### **6.4.2 Results and Discussions**

The results obtained (table 6.9) (figure 6.11a) for mobile phase extractions and (table 6.10) (figure 6.11b) for the MeOH-DCM 90:10 v/v revealed the presence of the same set of pyrolysis products in both extracts. This consisted of a mixture of EFV pyrolysis product 1, meconin, codeine, papaverine and EFV. The existence of these products amongst known pyrolysis products of opium has already been discussed in the previous chapters of this thesis.

All pyrolysis products except EFV and its pyrolysis product were detected in negligible amounts. This could probably be used to explain why only three products were detected with the GCMS; if the detected quantities were very little in the extracts, then they might have all decomposed upon injection in the GCMS (injection port) where temperatures were very high.

**Table 6.9 Results for the pyrolysis of mixture of EFV and opium extracted in the HPLC mobile phase.**

Pyrolysis Temperature ( in degrees Celsius)	HPLC EFV and opium	
	Mobile phase 25Mm buffer-ACN (53:47)	Retention time (minutes)
315	EFV product 1	1.01
	Codeine	1.72
	meconin	2.24
	Noscapine	2.79
	papaverine	8.12
	EFV	10.18
354	EFV product 1	1.01
	Morphine	1.35
	meconin	1.72
	Codeine	2.24
	Noscapine	2.79
	papaverine	8.12
	EFV	10.18

**Table 6.10 Results for the pyrolysis of a mixture of EFV and opium extracted in the MeOH-DCM 90:10**

Pyrolysis Temperature ( in degrees Celsius)	HPLC EFV and opium	
	Mobile phase 25Mm buffer-ACN (53:47)	Retention time (minutes)
315	EFV product 1	1.13
	morphine	1.34
	Meconin	1.71
	Codeine	2.33
	Noscapine	2.78
	papaverine	7.65
	EFV	10.23
354	EFV product 1	1.13
	Meconin	1.71
	Codeine	2.33
	Noscapine	2.78
	Papaverine	7.65
	EFV	10.23

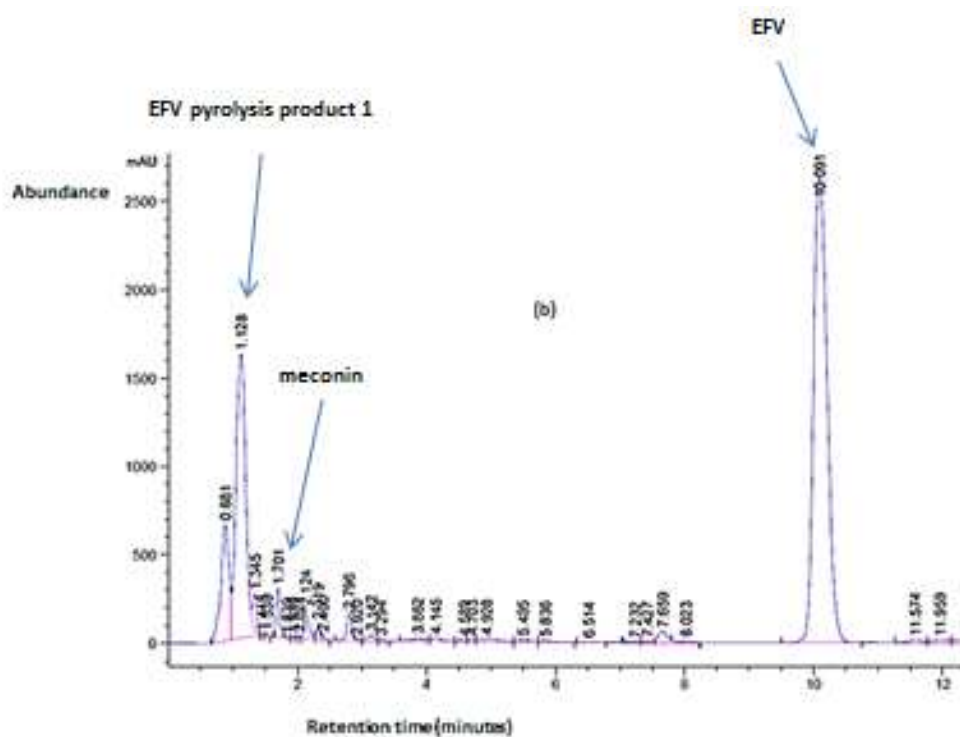
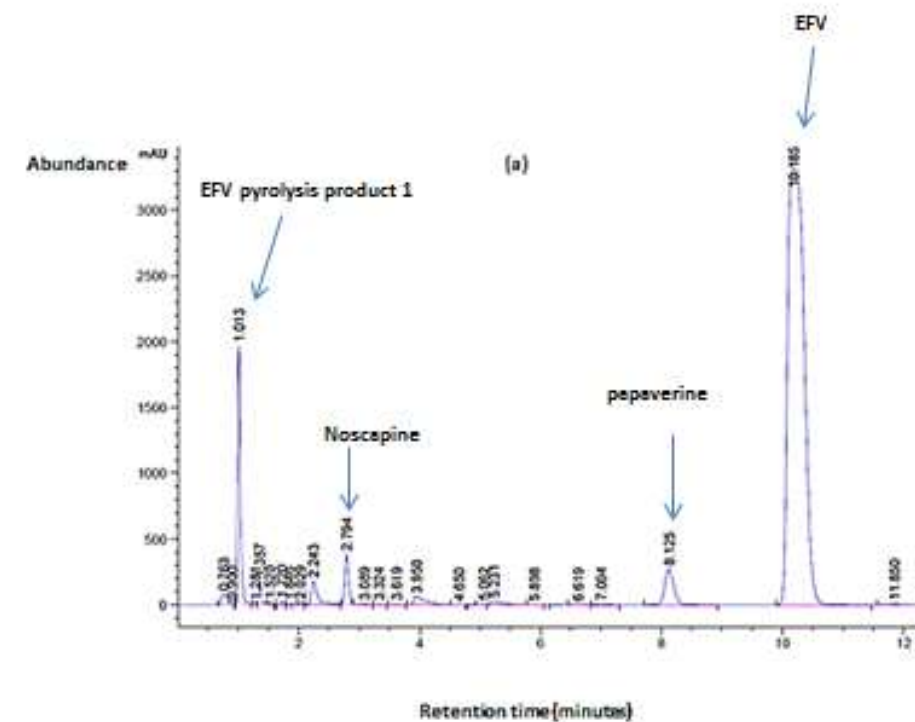
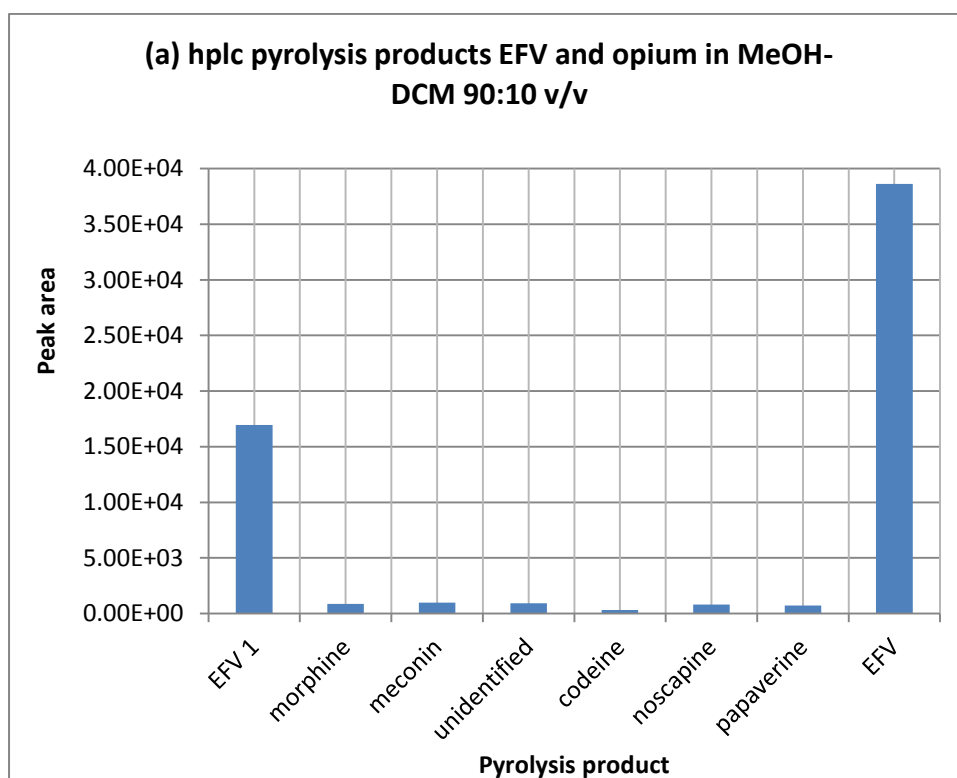
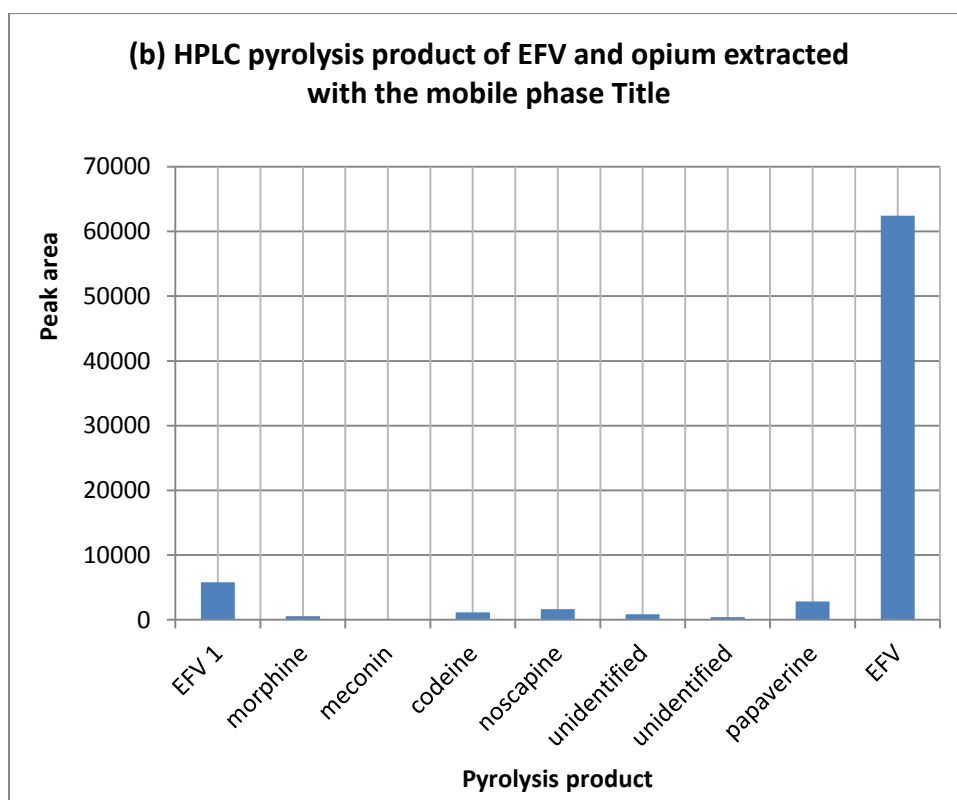


Figure 6.11 Chromatogram of pyrolysis products of opium extracted in the (a) mobile phase and (b) MeOH-DCM 90:10

The results obtained from the two extraction solvents were similar except for the absence of noscapine in the MeOH-DCM 90:10 v/v and meconin in the mobile phase.

Quantities of detected products were compared for the two solvents by drawing, plots of product vs peak area (figures 6.12a and 6.12b). The results obtained from both solvents revealed the detection of significant amounts of EFV and its pyrolysis product, and negligible amounts of opium alkaloids in bot. A tentative explanation for not detecting the alkaloids with the GCMS earlier was that there was a possibility of the EFV masking the opium from pyrolysing which may lead to only a small amount being extracted. These may have been completely destroyed by the GCMS during analysis. The HPLC on the other hand doesn't involve the application of heat hence their detection.





**Figure 6.12** Plot of quantities of pyrolysis products of EFV and opium extracted with the a) MeOH-DCM 90:10 and b) mobile phase

Figure 6.12a and b show various amounts of opium pyrolysis products detected with the HPLC. Both figures confirm the negligible quantities of detected pyrolysis products for all products except EFV and its pyrolysis product

## 6.5 Isolation of pyrolysis products of cannabis and analysis with the GCMS

### 6.5.1 Sample preparation

Six replicate cannabis samples were prepared following the protocol described in section 4.2.2.1. The samples were from finished forensic work. They were first homogenised by crushing them into fine powder using a mortar and pestle. 0.5g of the powdered material was weighed into 6 separate into tin cans. Each tin can was covered with a lid, heated to the desired temperature and removed from the hot plate. The covering lid was replaced with the one with prepared CAS strips (section 4.2.2.1) and incubated for 16 hours at 80<sup>0</sup>C to trap the pyrolysis products. The

products were trapped using four ACS in each of the six tin cans. Following this, the analysis sampling strategy in section 4.2.2.2 was applied. For GCMS analysis one of the four ACS strips was extracted with MeOH-DCM 90:10 and the other with MeOH-DCM 50:50.

## 6.5.2 Results and Discussions

The results obtained (Table 6.11 for both solvent extracts revealed the presence of cannabiniol, cannabidiol, clovanediol, terpenes and flavonoids. Typical chromatograms of pyrolysis products obtained from the pyrolysis of heroin at 354<sup>0</sup>C (extracted using MeOH-DCM 90:10 and MeOH-DCM 50:50) are displayed in figure 6.13 and 6.14 respectively.

**Table 6.11 Pyrolysis products of cannabis**

Pyrolysis Temperature ( in degrees Celsius)	Pyrolysis products of cannabis	
	DCM:MeOH 50:50	DCM:MeOH 10:90
315	Cannabinol Cannabidiol Clovanediol Humulene epoxide II Caryophyllene oxide Isoaromadenderene Terpineol	Cannabinol Cannabidiol Clovanediol Humulene epoxide II Caryophyllene oxide Isoaromadenderene Terpineol
358	Cannabinol Cannabidiol Clovanediol Humulene epoxide II Caryophyllene oxide Isoaromadenderene Terpineol	Cannabinol Cannabidiol Clovanediol Humulene epoxide II Caryophyllene oxide Isoaromadenderene Terpineol
398	Cannabinol Cannabidiol Limoneneoxide Isoaromadenderene Terpineol Humulene epoxide II Caryophyllene oxide Caryophyllene 1,2-dimethyl-2-octadecyl cyclohexane Alcohols	Cannabinol cannabicooumaronone Cannabidiol Clovanediol Humulene epoxide II Caryophyllene oxide Caryophyllene Isocaryophyllene Terpineol Fenchol

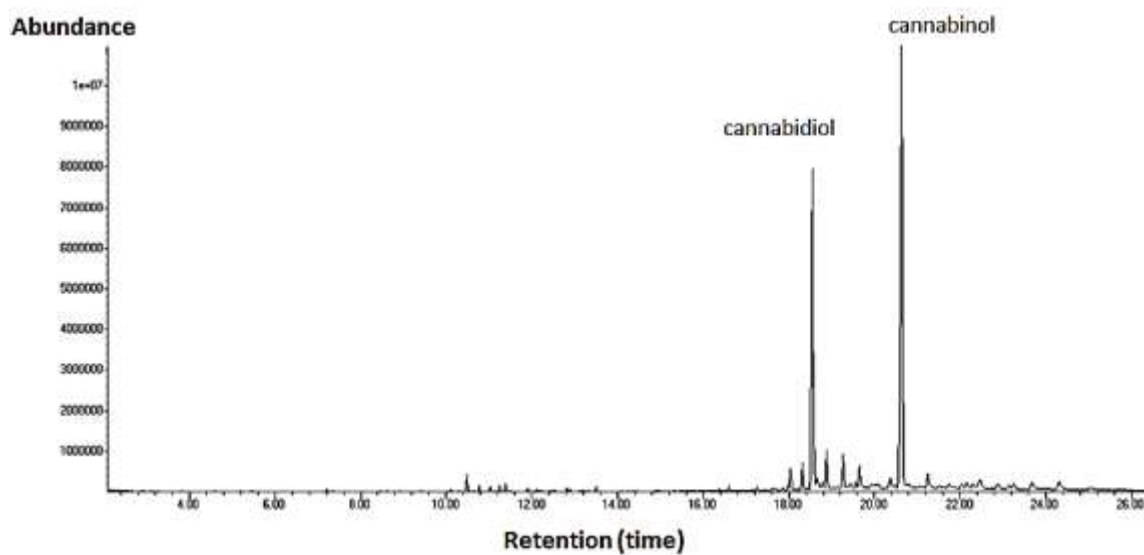


Figure 6.13 Chromatogram for pyrolysis products of cannabis extracted in MeOH-DCM 50:50 at 354°C

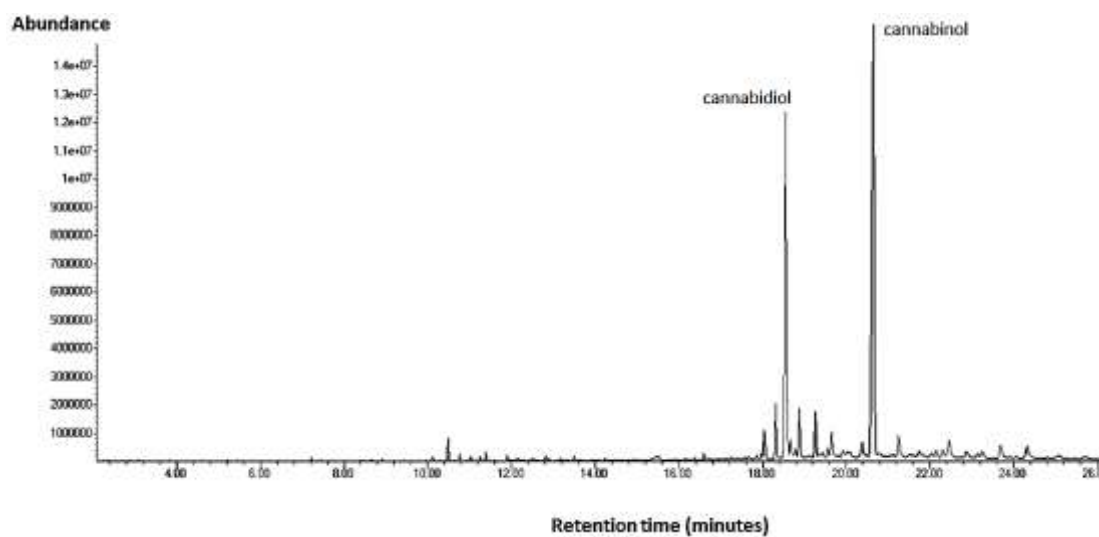


Figure 6.14 Chromatogram for the pyrolysis of cannabis extracted in MeOH-DCM 90:10 at 354°C

Cannabinoids are a characteristic class of substances unique to cannabis. More than 400 chemical compounds have been isolated from *Cannabis sativa* L., of which more than 80 have been reported as cannabinoids [194-195]. The most abundant are  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC or THC, the main psychoactive cannabinoid), cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG) and cannabichromene (CBC) [195-197].

THC was not however detected in the samples analysed, but its degradation product, cannabinol, was detected in quite a substantial amount. Cannabis samples with prolonged storage have been reported to have a diminished THC and an increased CBN content [197-199]. This probably explains why the THC was not detected in the used samples. Both cannabidiol and clovanediol are reportedly non-psychoactive, however medicinal benefits of cannabidiol have been reported [200-201]. Cannabis plants also contain other constituents commonly encountered in nature like terpenes, alkanes, flavonoids and nitrogenous compounds. Terpenes are reported to vary from one variety of cannabis to another and thought to be primarily responsible for differences in fragrance among the different strains [5, 201]. Few studies exist but terpenes are thought to be contributory to the distinctive smoking qualities and to the character of the "high" associated with smoking cannabis [202]. Some studies [203, 204] suggest that terpenes may somehow modify or enhance the physiological effects of the cannabinoids, however this has been only been assumed as hypothetical since there was no pre-clinical evidence at the time of these studies to support the hypothesis and also, no clinical trials on this subject have been carried out [204].

### **6.5.3 Method repeatability test for pyrolysis products of cannabis**

The repeatability of the analytical methods (pyrolysis generation and desorption) method for cannabis pyrolysis products using the HPLC was evaluated through calculation of %RSDs from peak areas. Average peak areas for all the 6 replicate injections from the 6 ACS in sample can 1 were used to represent the within-can analytical repeatability. The results obtained for samples pyrolysed at 354°C from



the mobile phase are presented in table 6.12 while those from the MeOH-DCM (90:10) are in table 6.13. 3 Both results revealed %RSD values of  $\leq 5\%$ , indicating very good repeatability.

The repeatability of inter-can results was also evaluated. This was obtained from %RSDs values calculated from average peak areas of pyrolysis products generated from the 6 injections in each of the 6 sample cans (n=36). The results obtained using the mobile phase are presented in table 6.14 while those for the MeOH-DCM 90:10 are in table 6.15. Both results revealed %RSD values of  $\geq 5\%$ , indicating that repeatability was poorer as expected for this kind of work. Plots of peak area versus pyrolysis (figures 6.15 and 6.16) also revealed variations in the quantities of pyrolysis products.

## 6.6 Isolation of pyrolysis products of cannabis and analysis with the HPLC

### 6.6.1. Sample preparation

Opium samples used for the HPLC analysis were the two remaining ACS strips from the four prepared and detailed in section 6.1.1. In this case two of the ACS strips were analysed using the HPLC where one was extracted using MeOH-DCM 90:10 and the second extracted using the mobile phase. The samples were obtained from finished forensic work.

### 6.6.2 Results and Discussions

The results obtained from samples extracted with the mobile phase (table 6.16) (figure 6.17a) and MeOH-DCM 90:10 (table 6.17) (figure 6.17b) revealed the presence of same pyrolysis products being cannabinal, cannabidiol and other unidentified impurities. The impurities were thought to be possibly cannabis terpenes or flavonoids [33, 39] as these had been detected previously in samples analysed with the GC-MS.

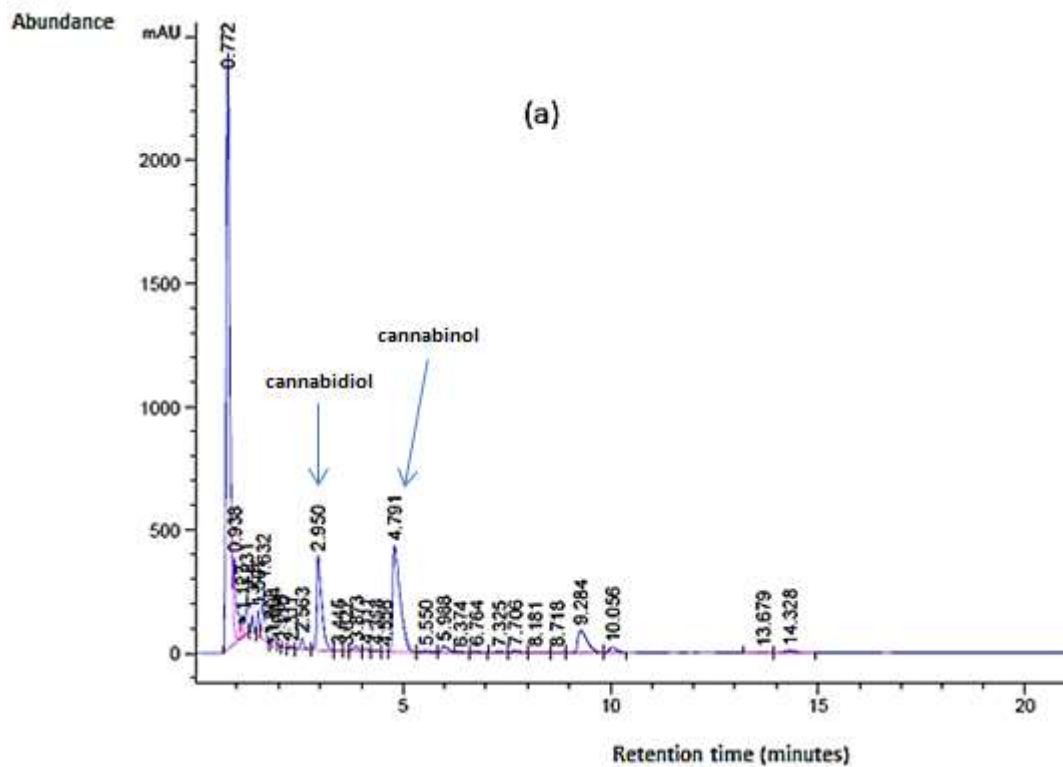
The main psychoactive substance of cannabis,  $\Delta^9$  THC, was not detected from both extractions but its degradation product, cannabinal was present; its absence in the samples is indicative that the samples used have been on storage for a long time.

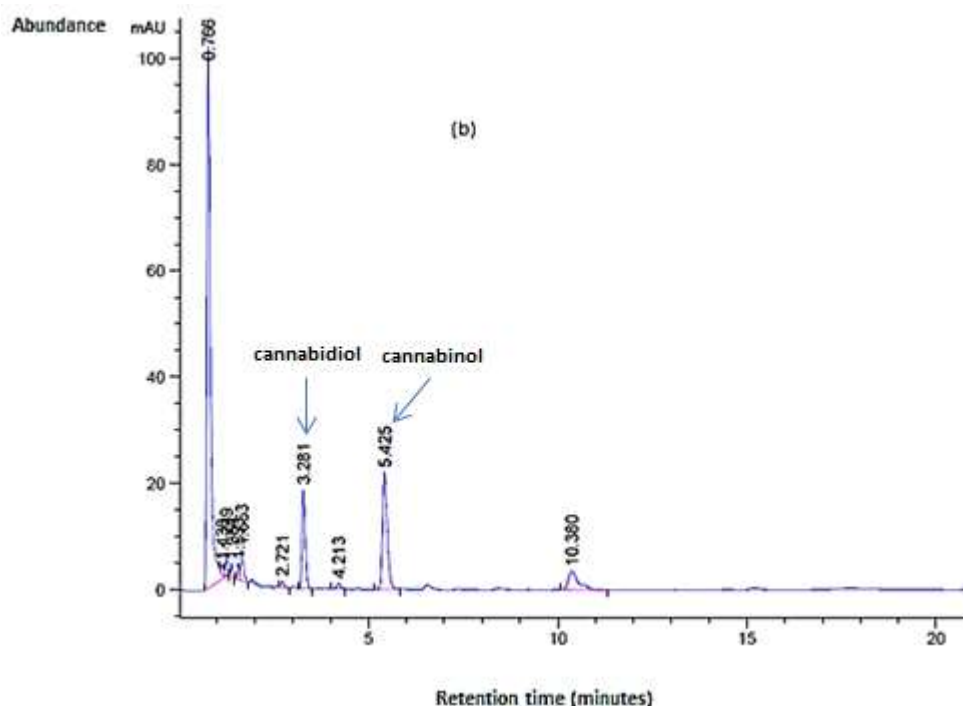
**Table 6.16 Pyrolysis products of cannabis extracted in the mobile phase**

Pyrolysis Temperature ( in degrees Celsius)	HPLC Cannabis	
	Mobile phase 25Mm buffer-ACN (53:47)	Retention time (minutes)
315	Cannabinal	4.79
	Cannabidiol	2.95
358	Cannabinal	5.43
	Cannabidiol	3.28
398	Cannabinal	4.79
	Cannabidiol	2.95

**Table 6.17 Pyrolysis products of cannabis extracted in the MeOH-DCM 90:10**

Pyrolysis Temperature ( in degrees Celsius)	HPLC Cannabis	
	Mobile phase 25Mm buffer-ACN (53:47)	Retention time (minutes)
315	Cannabinol	5.43
	Cannabidiol	3.28
358	Cannabinol	5.43
	Cannabidiol	3.28
398	Cannabinol	5.43
	Cannabidiol	3.28





**Figure 6.17 Chromatogram of pyrolysis products of cannabis extracted in the HPLC mobile phase (a) and MeOH-DCM 90:10**

### 6.6.3 Method repeatability test for pyrolysis products of cannabis using the HPLC

The repeatability of the analytical methods (pyrolysis generation and desorption) method for cannabis pyrolysis products using the HPLC was evaluated through calculation of %RSDs from peak areas. Average peak areas for all the 6 replicate injections from the 6 ACS in sample can 1 were used to represent the within-can analytical repeatability. The results obtained for samples pyrolysed at 354<sup>o</sup>C from the mobile phase are presented in table 6.18 while those from the MeOH-DCM (90:10) are in table 6.19 (appendix B). Both results revealed %RSD values of  $\leq 5\%$ , indicating very good repeatability.

The repeatability of inter-can results was also evaluated. This was obtained from %RSDs values calculated from average peak areas of pyrolysis products generated from the 6 injections in each of the 6 sample cans (n=36). The results obtained using the mobile phase are presented in table 6.20 while those for the MeOH-DCM 90:10 are in table 6.21. Both results revealed %RSD values of  $\geq 5\%$ , indicating that repeatability was poorer as expected for this kind of work. Plots of peak area versus

pyrolysis (figures 6.18 and 6.19) (appendix A) also revealed variations in the quantities of pyrolysis products.

## **6.7 Isolation of pyrolysis products of EFV and cannabis, and analysis with the GCMS**

### **6.7.1 Sample preparation**

Six replicate samples of EFV and cannabis (from finished forensic work), were prepared by weighing 0.5g of each drug, mixing the two drugs together and then placing the mixture with 6 separate sampling tin cans. The mixture was heated to 354<sup>0C</sup> and pyrolysis products extracted according to the protocol described previously in section 4.2.2.1. As previously discussed pyrolysis products were trapped using four ACS in each of the six sampling cans where one of the four ACS strips was extracted with MeOH-DCM 90:10 and the other with MeOH-DCM 50:50 with the final two being reserved for HPLC analysis.

### **6.7.2 Results and Discussions**

The results obtained revealed the presence of EFV and its pyrolysis product 1 (table 6.22) across the 6 tin cans for both solvent extracts. Typical chromatograms for pyrolysis products obtained from the pyrolysis of methamphetamine at 354<sup>0C</sup> (extracted using MeOH-DCM 90:10) is displayed in figure 6.20 4.1. The EFV was found to produce the expected pyrolysis products when it heated with cannabis but its presence in the mixture was discovered to mask the pyrolysis of the cannabis. The presence of cannabis on the other hand did not affect the expected pyrolysis pattern for EFV.

**Table 6.22 Pyrolysis products of EFV and cannabis extracted in the MeOH-DCM 90:10 at 354<sup>0</sup>C**

ACS extracting solvent	Pyrolysis Temperature (°C)		
	315	354	396
<b>MeOH:DCM 50:50</b>	EFV EFV pyrolysis product	EFV EFV pyrolysis product	EFV EFV pyrolysis product 1
<b>MeOH:DCM 90:10</b>	EFV EFV pyrolysis product	EFV EFV pyrolysis product	EFV EFV pyrolysis product

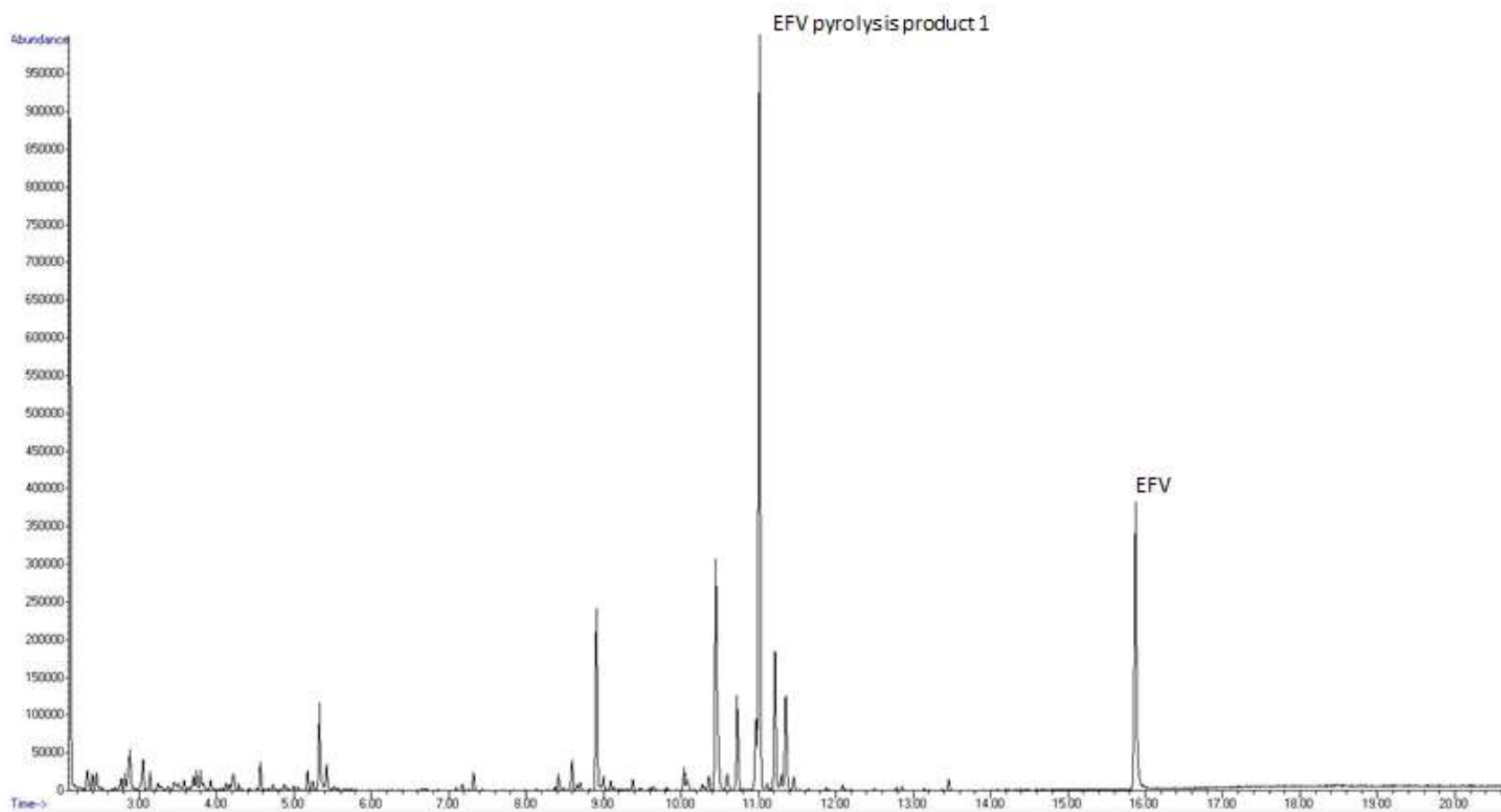


Figure 6.20 Chromatogram for Pyrolysis products of EFV and cannabis extracted in MeOH 90:10 at 354°C

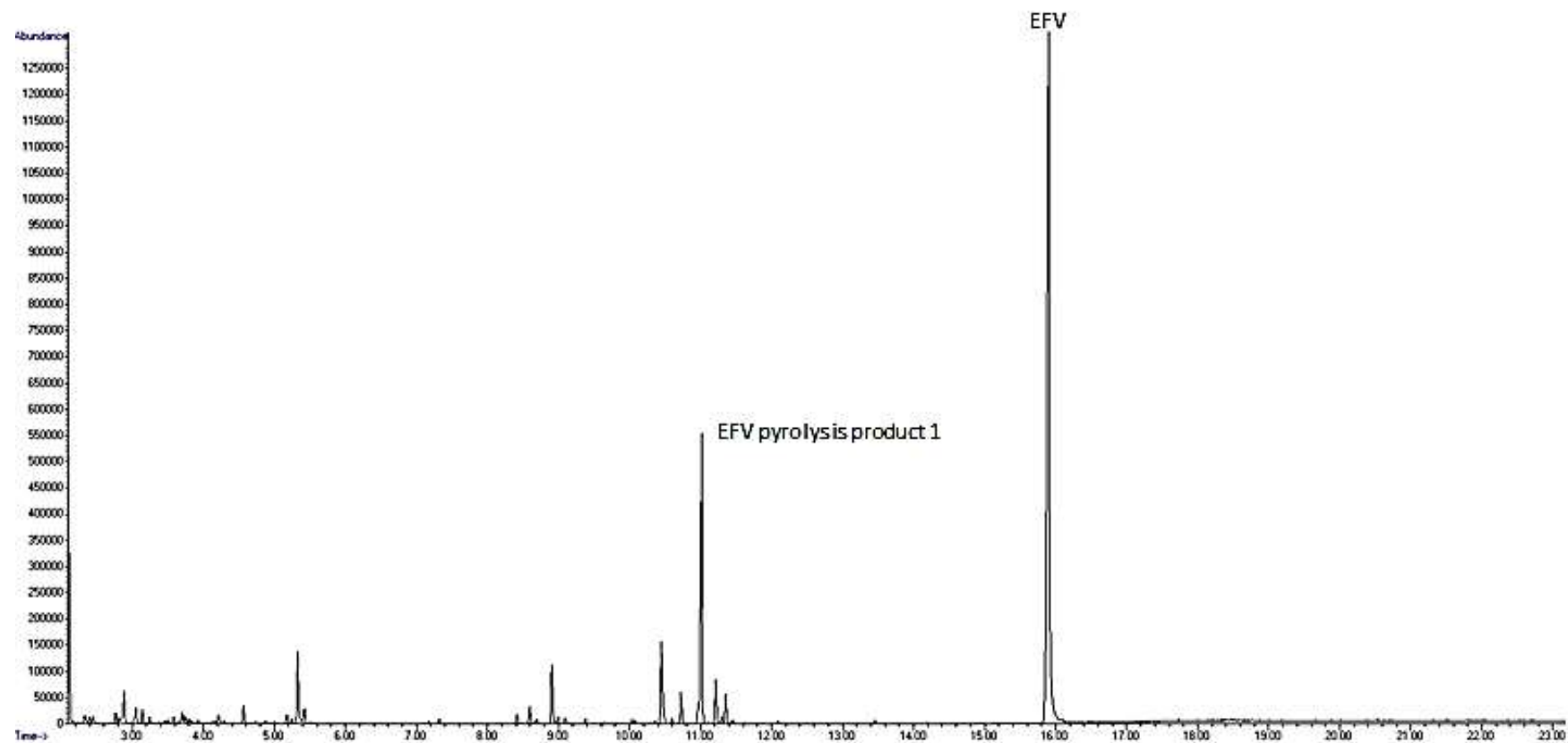


Figure 6.21 Chromatogram for pyrolysis products of EFV-cannabis extracted in the MeOH-DCM 50:50 at 354°C



The repeatability of the method for desorbed pyrolysis products of EFV and cannabis using the GCMS was not performed because the repeatability of the two detected products had been performed when EFV was heated with other drugs, where the within can repeatability were found to be excellent while the inter can results indicated that repeatability was not achievable.

## **6.8 Isolation of pyrolysis products of cannabis and EFV and analysis with the HPLC**

### **6.8.1 Sample preparation**

Six replicate samples of EFV and heroin from finished case work were prepared by weighing 0.5g of each drug, mixing together and placing the mixture into a sample can. 6 mixed samples where prepared in this way. The mixture was heated to 354<sup>0</sup>C and pyrolysis products extracted according to the protocol described previously.

### **6.8.2 Results and discussions**

The results obtained (Tables 6.23) (figures 6.21a) for mobile phase extractions and (Table 6.23) (figure 6.21b) for MeOH-DCM 90:10 revealed the presence of EFV and EFV product 1.

**Table 6.23 Pyrolysis products of EFV-cannabis extracted in the HPLC mobile phase**

Pyrolysis Temperature ( in degrees Celsius)	HPLC Cannabis	
	Mobile phase 25Mm buffer-ACN (53:47)	Retention time (minutes)
354	EFV pyrolysis product 1 EFV	1.431 10.499

**Table 6.24 Pyrolysis products of EFV-cannabis extracted in MeOH-DCM 90:10**

Pyrolysis Temperature ( in degrees Celsius)	HPLC Cannabis	
	Mobile phase 25Mm buffer-ACN (53:47)	Retention time (minutes)
354	EFV pyrolysis product 1 EFV	1.431 10.260

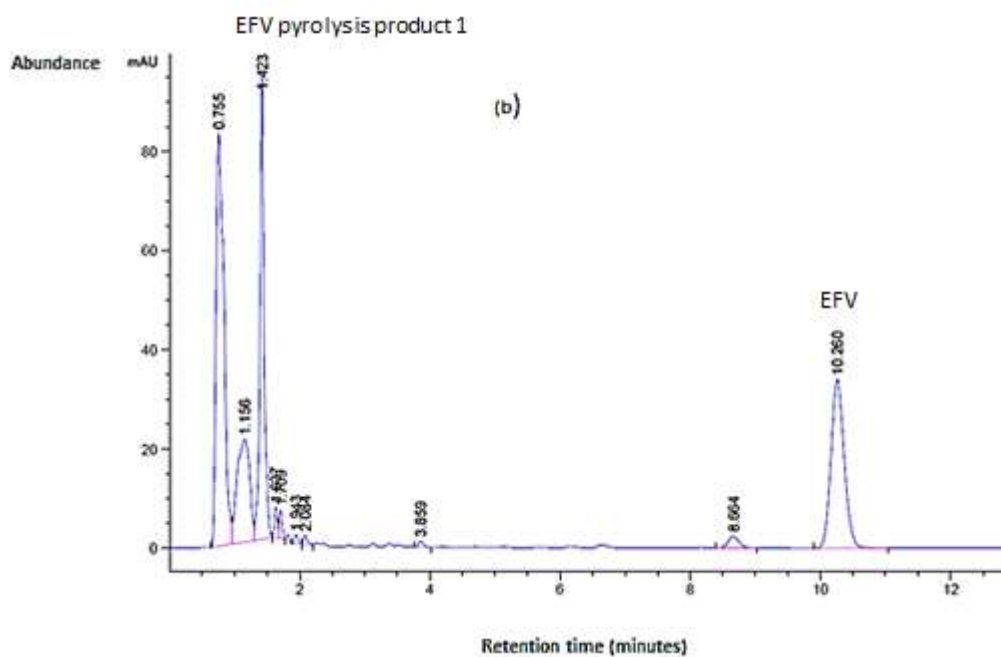
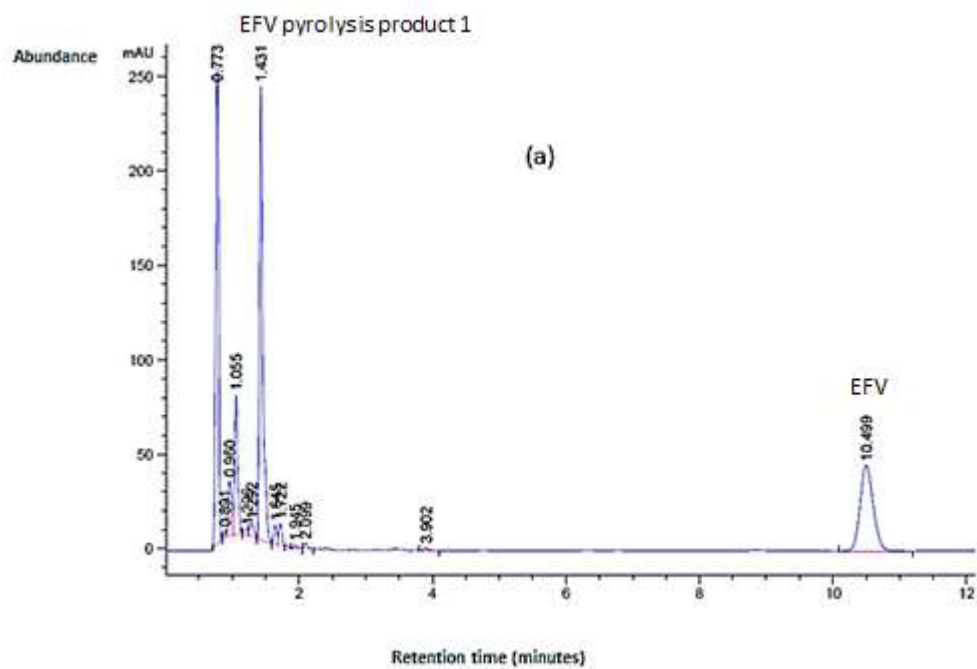


Figure 6.22 Chromatogram of pyrolysis products of EFV and cannabis extracted in the (a) mobile phase and (b) MeOH-DCM 90:10

The results obtained from analysis of samples extracted using MeOH-DCM 90:10 solvent and the mobile phase revealed the presence of similar pyrolysis products. The two results HPLC also correlate those for the GCMS. Based on the results it was concluded that when EFV is heated with cannabis, it will mask/suppress its pyrolysis but cannabis does not alter the pyrolysis of EFV.

The repeatability of the method for desorbed pyrolysis products of EFV and cannabis using the HPLC was not performed because the repeatability of EFV and its pyrolysis product was already known. This was based on results from similar experimental work where EFV was heated with other drugs and the within can repeatability were found to be excellent while the inter can results indicated that repeatability was not achievable.

#### **6.9 Summary results for the pyrolysis of EFV, opium and cannabis**

The pyrolysis of opium on its own produced typical alkaloids of papaverine, noscapine, thebaine, morphine and codeine as well as the breakdown products of noscapine. However, heating a mixture of EFV and opium produced only the EFV and its pyrolysis product. The same scenario was observed with cannabis, which upon heating with EFV, the same pyrolysis products were also obtained but not the cannabinoids as previously observed when cannabis was heated on its own. The two results reveal that EFV prevents the pyrolysis the pyrolysis of the two plant based drugs. It can therefore be safely concluded that when a mixture of EFV and one of the two natural drugs is smoked for abuse, pyrolysis products will comprise mostly of the EFV and its pyrolysis product.

## CHAPTER 7: DISCUSSIONS

### 7.1 Summary of results

The new South African street drug nyaope drug is allegedly comprised of a mixture of illicit substances including methamphetamine, amphetamine, heroin, opium, cannabis and the anti-retroviral drug EFV as the main ingredient. Available information reveals that the drug is mainly abused through smoking. Nyaope was criminalised in 2014, even though there are no documented analytical methods for the identification of its smoked products, especially EFV. Analytical methods for the analysis of pyrolysis products of most illicit drugs which are alleged to be components of nyaope have already been developed but none of these includes EFV. As a result, the development of a single analytical methodology capable of identifying smoked products of EFV and all other alleged components of nyaope was necessary. Through this research, an analysis methodology for the analysis of smoked products of nyaope including EFV, was developed and validated for the GCMS and HPLC techniques. This bridged the gap for EFV for which, now as a controlled substance, a clear analytical methodology was required.

Literature reveals GCMS and HPLC as commonly used techniques for the identification of pyrolysis products. To make it a more powerful technique, the LC may be paired with other techniques such as MS, UV-Vis, DAD, fluorescence spectroscopy and others.

The GCMS is a discriminatory technique and capable of identifying volatile pyrolysis products of the nyaope but since it is destructive, the collection of these products for further studies is impossible. Unlike the GCMS technique, HPLC allows for the identification of both non-volatile and volatile pyrolysis products. The products can be collected in the mobile phase as they elute from the column, purified and studied further.

Many laboratories include derivatisation as part of their sample preparation for GC analysis. It is a process by which a compound is chemically changed, producing a new compound that has properties more responsive to a particular analytical

method [89]. Many analytical methods exist for the derivatisation and analysis of some of the alleged components of nyaope like heroin and methamphetamine [205, 206]. Street heroin may contain bulking agents which possess functional groups which, if not derivatised prior to analysis with the GC, are likely to exhibit poor peak shape and poor separation. One of such bulking agents for heroin is acetaminophen (commonly known as paracetamol), which has both the hydroxyl and carbonyl functional groups. Like paracetamol, methamphetamine and amphetamine possess polar amino groups, which can cause them separate poorly if not derivatised. Even though necessary at times, derivatisation can also complicate the interpretation of the analytical data because the resultant reaction may introduce impurities, uncertainty on the completeness of the analytes' conversion, and other interference factors.

Derivatisation of samples was not considered for this research because the main aim was to isolate the pyrolysis products of EFV; an analysis which had never been performed previously. Based on the chromatographic profile obtained, this did not seem to have affected the separation of EFV as very resolved peaks were obtained. Heroin and ATS drugs were affected by non derivatisation as poorly shaped and separated peaks for methamphetamine, amphetamine and paracetamol were obtained. It is not known as to what will happen when EFV is derivatised, but since it has both the carbonyl and hydroxyl group, it might yield more than the pyrolysis product.

Separation column efficiency can be improved in many ways. Factors that influence column efficiency and how they can be improved are elaborated under the Van Deemter equation theory. Chromatography literature reveals that length of the GC column is proportional to the number of theoretical plates and that increasing length will enhance the separation resolution by roughly 1.4 times [89]. The length of the separation column used for this research was 15 meters. It will be interesting to find out whether the analysis of pyrolysis products of EFV on a longer column will still reveal only one pyrolysis product.

The new method was used to investigate the effect of efavirenz on the pyrolysis of other alleged components of the nyaope drug mixture (amphetamine, methamphetamine, heroin, opium and cannabis) and vice versa. This was performed by heating each drug on its own followed by mixing it with EFV and heating the mixture. A summary of results are presented in table 7.1.

**Table 7.1. Summary of results obtained from the pyrolysis of EFV and other drugs alleged to be in the Nyaope mixture using both the GCMS and HPLC**

<b>Alleged Nyaope street drug component</b>	<b>Pyrolysis of the drug material on its own</b>	<b>Pyrolysis of drug material with EFV</b>
Amphetamine	√	√
Methamphetamine	√	√
Heroin	√	√
Opium	√	EFV + pyrolysis product 1
Cannabis	√	EFV + pyrolysis product 1

According to results obtained, the pyrolysis of each drug on its own yielded the expected pyrolysis products. EFV was found to produce only one pyrolysis product when heated on its own and in all drug mixtures. The presence of EFV was found not to have influence the expected pyrolysis processes of amphetamine type stimulant drugs and of heroin. However the presence of EFV in cannabis and opium mixtures was discovered to have prevented the pyrolysis of the two drugs. Opium and cannabis are both drugs derived from natural materials; it is not known why this happened but one possible answer could be the length time at which the samples were heated. It is not known what could have happened if the samples were heated a bit longer; but it will be interesting to see if this would still yield only pyrolysis product.

In forensic science, ACS is commonly used to trap traces of ignitable liquids during fire investigations, but recently its application has since been extended to trapping volatile substances from human remains [207, 208] and for trapping impurities from volatile chemicals during clandestine drug laboratory investigations [209]. While other trapping media are available and have been used successfully [210-212], the robustness and its successful use as a trapping media made it a preferred choice for use in the study given that the method was developed for Africa where the success of an analysis depends on other factors like the availability of finances and also electrical energy to operate instruments.

When used for this research, the ACS was able to trap pyrolysis products which had been reported before with the use other trapping media [213]. This successful use of ACS as a trapping medium for pyrolysis products of EFV and other alleged components of the nyaope drug mixture makes it a possible cheaper alternative for the adsorption of pyrolysis products of illegal drugs, particularly those in the nyaope mixture.

The repeatability of the method for pyrolysis products of EFV, other drugs and EFV-drug mixtures was evaluated; %RSD results of peak areas for pyrolysis products from each tin can were calculated and used to represent analytical repeatability of products within each can (within-can repeatability). Analytical reproducibility was calculated using %RSD for average peak areas ( $n=6$ ) of pyrolysis products detected within each can compared across all 6 tin cans to generate the between can reproducibility ( $n=36$ ). The results are summarised in table 7.2 and 7.3 below. Calculated %RSDs for the analysis of pyrolysis products within a can were all  $\leq 5\%$ , indicating excellent repeatability while those for inter-can repeatability were all  $\geq 5\%$ , indicating that repeatability was more challenging. Results for inter-can repeatability were expected due to the nature of the pyrolysis experiment.



**Table 7.2 Summary of GCMS results for analytical repeatability of pyrolysis products of EFV and other alleged components of nyaope street drug**

<b>Alleged nyaope component</b>	<b>%RSD results for within-can analytical repeatability in MeOH-DCM 50:50</b>	<b>%RSD results for inter-can analytical repeatability in MeOH-DCM 50:50</b>	<b>%RSD results for within-can analytical repeatability in MeOH-DCM 90:10</b>	<b>%RSD results for inter-can analytical repeatability in MeOH-DCM 90:10</b>
Methamphetamine	0.8-5	64-150	0.6-5	37-87
EFV and methamphetamine	0.8-6	12-108	0.002-0.8	37-95
Amphetamine	0.1-6	36-132	0.02-2	86-180
EFV and amphetamine	0.1-6	36-133	0.01-2	18-150
Heroin	0.02-3	58-256	0.002-2	47-166
EFV and heroin	0.1-5	75-198	0.27-4	48-102
Opium	0.4-0.6	194-239	0.004-0.3	182-237
Cannabis	0.8-2	40-132	0.01-2	28-245

**Table 7.3 Summary of HPLC results for analytical repeatability of EFV and methamphetamine pyrolysis products**

<b>Alleged Nyaope component</b>	<b>%RSD results for within-can analytical repeatability in mobile phase</b>	<b>%RSD results for inter-can analytical repeatability in mobile phase</b>	<b>%RSD results for within-can analytical repeatability in MeOH-DCM 90:10</b>	<b>%RSD results for inter-can analytical repeatability in MeOH-DCM 90:10</b>
Methamphetamine	0.01-4	6 -75	0.14-2	17-61
EFV and methamphetamine	0.7-5	24-50	0.04-0.4	10-80
Amphetamine	0.4-2	2-118	0.0023-3	47-121
EFV and amphetamine	0.2-3	35-69	0.008-2	100-257
Heroin	0.06-2	14-97	0.20-2	14-97
EFV and heroin	0.1-5	75-198	0.3-4	48-102
Opium	0.4-0.6	132-265	0.004-0.3	182-237
Cannabis	0.5-0.8	143-186	0.2-0.2	32-53

Comparison of results obtained from samples extracted using the MeOH-DCM 90:10 for both the HPLC and the GCMS were performed for each EFV drug mixture. The results obtained (table 7.1) were used to draw conclusions about the suitability of each technique for the analysis of alleged components of the nyaope drug mixture following extractions with MeOH-DCM 90:10.

**Table 7.4** Number of pyrolysis products obtained using the MeOH-DCM 90:10 for both the GCMS and HPLC

Alleged nyaope street drug component	Number of detected pyrolysis products in MeOH-DCM 90:10 (GCMS)		Number of detected pyrolysis products in MeOH-DCM 90:10 (HPLC)	
	Single drug	EFV-drug mixture	Single drug	EFV-drug mixture
Methamphetamine	7	9	7	8
Amphetamine	6	11	4	6
Heroin	6	6	3	6
Opium	6	2	3	2
cannabis	8	2	2	2

Based on the highest number of pyrolysis products obtained between the two techniques, it can safely be concluded that the method recommended for the analysis of alleged components of the nyaope street drug is the GCMS method. The analytical repeatability factor could not be used to discriminate between the two methods since it had already been established that the two techniques were capable of producing excellent within- can analytical repeatability results.

Results obtained from both MeOH-DCM solvent ratios for the GCMS analysis (table 7.2) were compared and used to determine the most suitable solvent (between the two) for extracting pyrolysis products of alleged components of nyaope drug mixture for analysis with the GCMS.

**Table 7.5 Total number of pyrolysis products obtained from the GCMS extracted using MeOH-DCM 50:50 and 90:10**

Alleged nyaope street drug component	Number of detected pyrolysis products in MeOH-DCM 90:10 (GCMS)		Number of detected pyrolysis products in MeOH-DCM 50:50 (GCMS)	
	Single drug	EFV-drug mixture	Single drug	EFV-drug mixture
Methamphetamine	7	9	9	13
Amphetamine	6	11	9	11
Heroin	6	6	8	8
Opium	6	2	6	2
cannabis	8	2	7	2

According to the results obtained, the highest number of pyrolysis products was obtained from extractions using MeOH-DCM 50:50. This therefore makes it the most suitable solvent for the extraction of pyrolysis products of alleged components of the nyaope drug mixture.

## 7.2 CONCLUSIONS

A methodology for routine isolation and analysis of pyrolysis products of Efavirenz in its mixture with other alleged components of the Nyaope street drug was developed and validated for the GCMS and HPLC techniques. The research has bridged the gap for EFV for which, now as a controlled substance, a clear analytical methodology was required. EFV was found to produce only one pyrolysis product which did not interfere with the pyrolysis processes of amphetamine type stimulant drugs and heroin. The pyrolysis of EFV with cannabis and opium however only yielded EFV and its pyrolysis product; no cannabinoids or opium alkaloids were detected. The successful use of ACS as a trapping medium for pyrolysis products of EFV and other components of the Nyaope drug mixture confirms it as a possible low cost option for the adsorption of pyrolysis products of illegal drugs in the Nyaope drug mixture.

## 7.2. RECOMMENDATIONS FOR FUTURE WORK

Now that it has been confirmed that the pyrolysis of EFV produces a pyrolysis product that can be detected and quantified, it is recommended that further studies on this product together with determination of its correct chemical structure be conducted using more accurate techniques such as High Resolution Mass Spectroscopy (HRMS) and Nuclear Magnetic Resonance Spectroscopy (NMRS). Once this has been achieved the product can be synthesised and its neuropharmacology studied further.

According to the study by Gatch et al [69], it has been confirmed that the behavioural pharmacology produced by EFV in mice is very similar to that produced by Lysergic acids (LSDs); receptor binding assays could be performed using the pyrolysis product and known LSD receptors. The studies could potentially determine if the product is abused for the same or similar hallucinogenic-type effects.

The studies of the pyrolysis product(s) could also be studied to see if they correlate with some of the alleged EFV medication-induced side effects described as adverse neuropsychiatric events in the literature such as anxiety, depression and hallucinations [70, 214].

Derivatisation of samples was not performed during this study; it is recommended that EFV samples and those containing EFV in mixtures with other drugs (used to mimic the nyaope drug mixture) be derivatised in future and the results obtained compared with those obtained during this study. This is important for EFV, which, given its chemical structure; might yield more than one pyrolysis products upon derivatisation.

Since the study involved the use of substances that mimicked the nyaope street drug, it is recommended that the method be extended to the analysis of real life cases.

The use of a proper cigarette smoking machine or a smoking simulator is recommended to allow for further studies of the pyrolysis products of EFV.

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**Appendix A: Chromatic profiles of pyrolysis products of different drugs alleged to be components of nyaope drug mixture**

Chromatographic overlays of pyrolysis products of amphetamine, heroin, opium, and cannabis are displayed in figures 4.43 –figure 4.

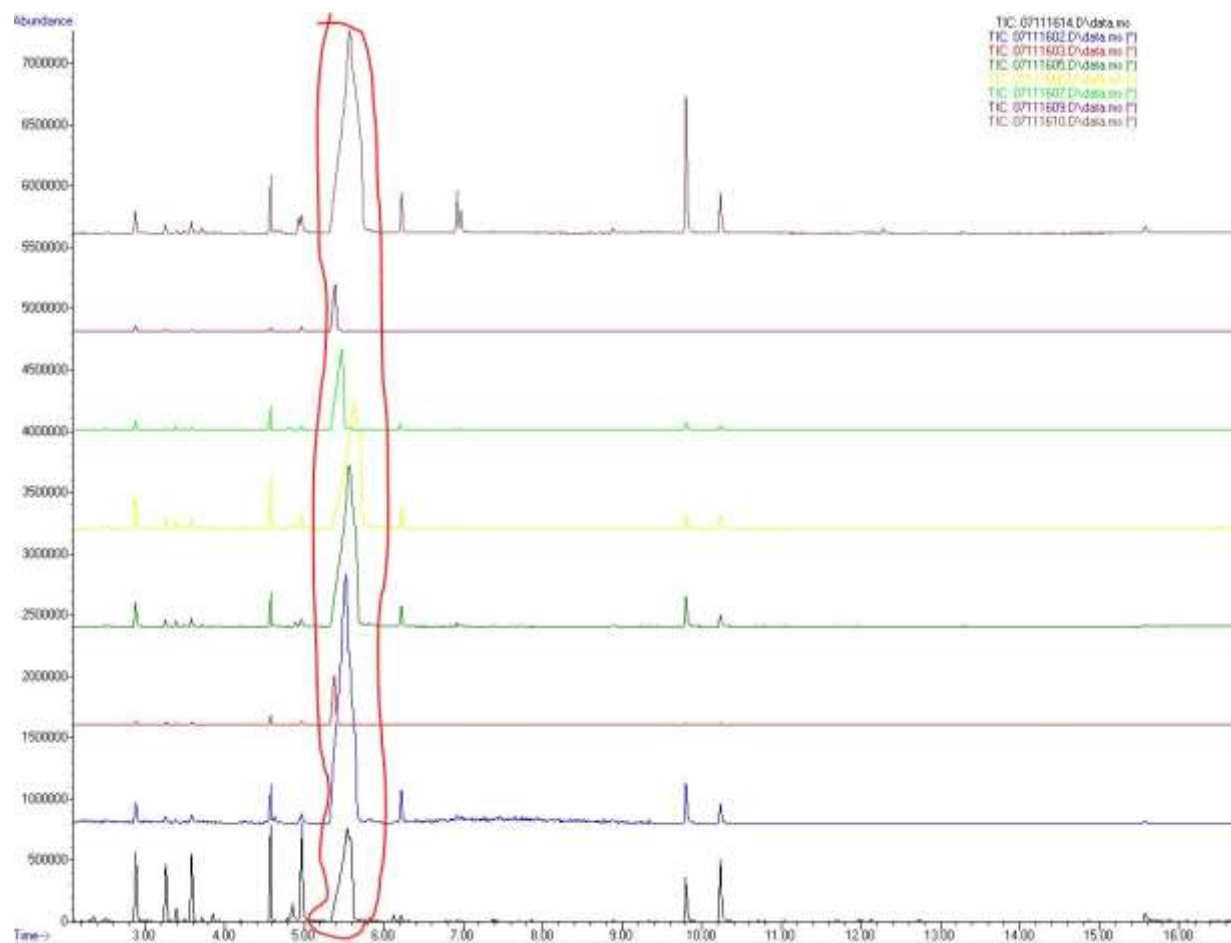


Figure 4.43 Overlay of chromatographic profiles of amphetamine pyrolysis products extracted in MeOH-DCM 50:50 at 3540C. A large amphetamine peak is highlighted

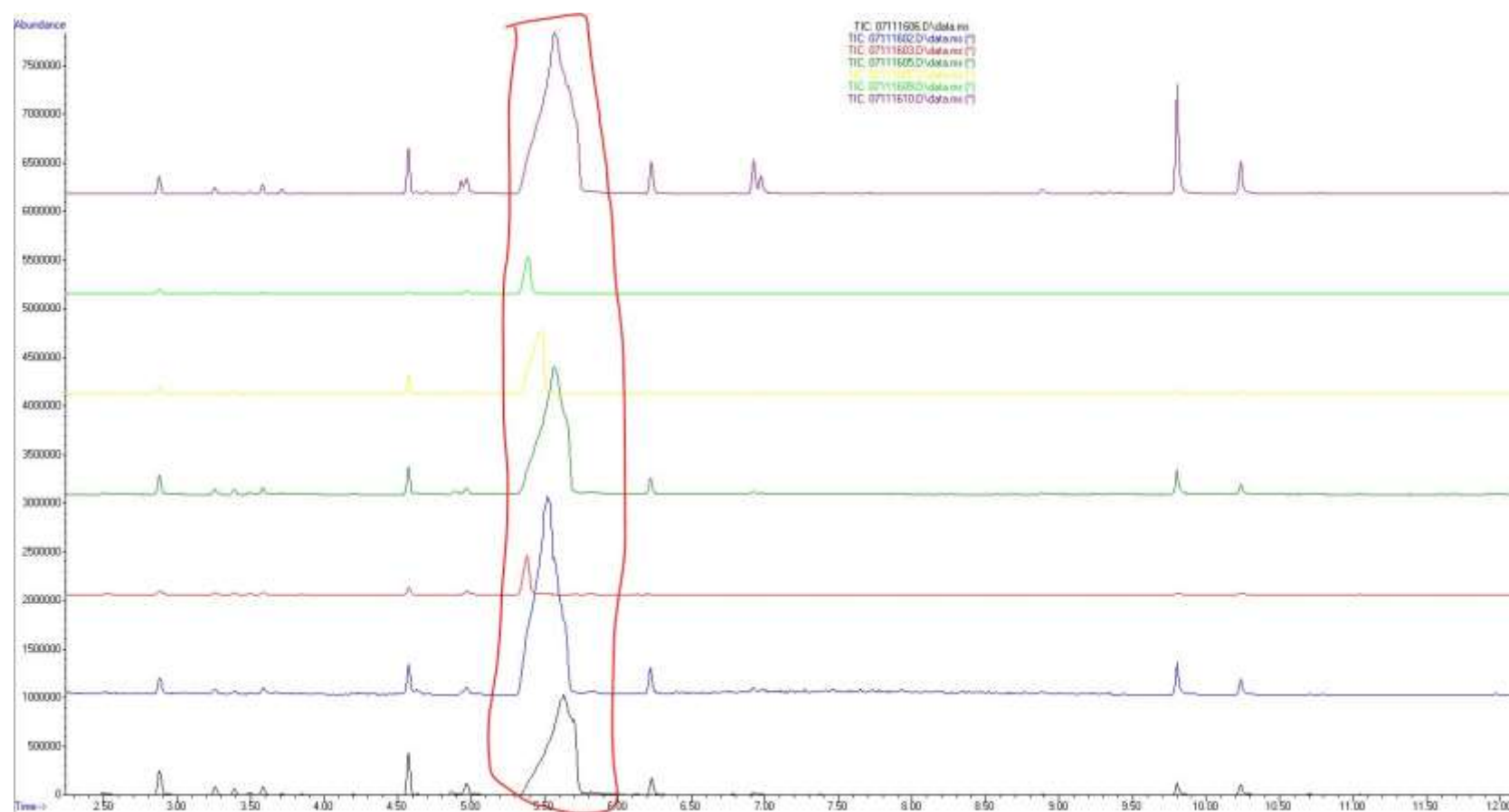


Figure 4.44 Overlay of chromatographic profiles of pyrolysis products of amphetamine extracted in MeOH-DCM 90:10 at 354<sup>0</sup>C. A large amphetamine peak is shown.

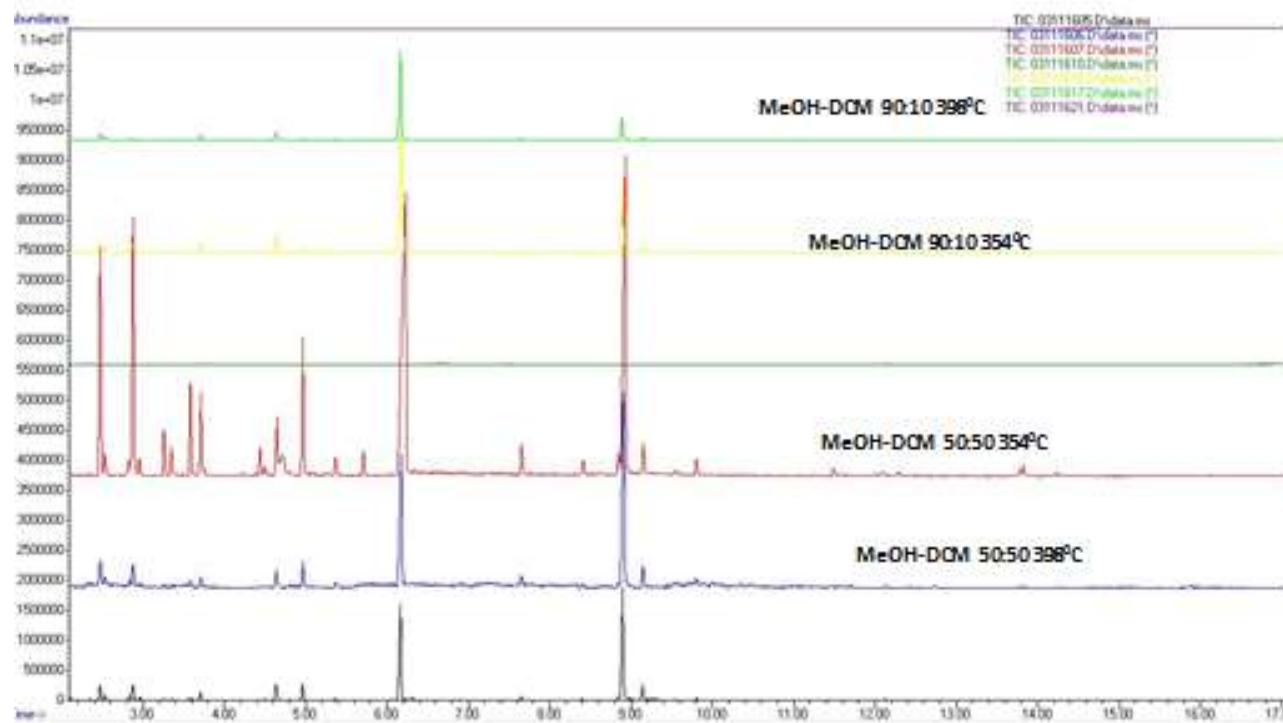
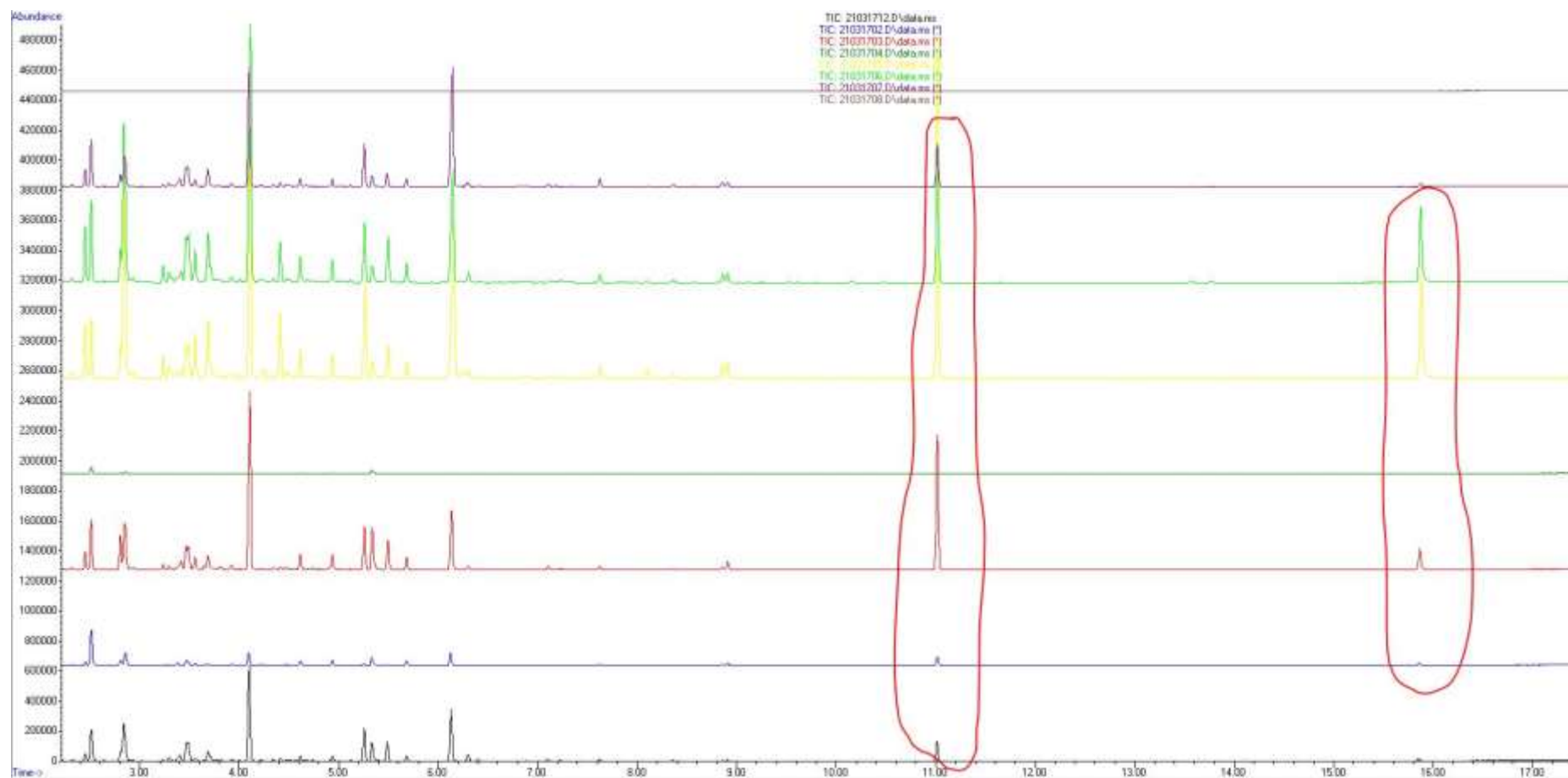


Figure 4.45 Overlay of chromatographic profiles of pyrolysis products of amphetamine extracted in MeOH-DCM 50:50 and 90:10 at 354°C and 398°C



**Figure 4.50** Overlay of chromatographic profile of pyrolysis products of EFV-amphetamine extracted with MeOH-DCM 50:50. EFV pyrolysis product 1 is shown at 11minutes and EFV at 16 minutes





Figure 4.51 Overlay of chromatographic profiles of pyrolysis products of EFV-amphetamine in MeOH-DCM 90:10 at 3540c, showing EFV pyrolysis product 1 peak at 11 minutes

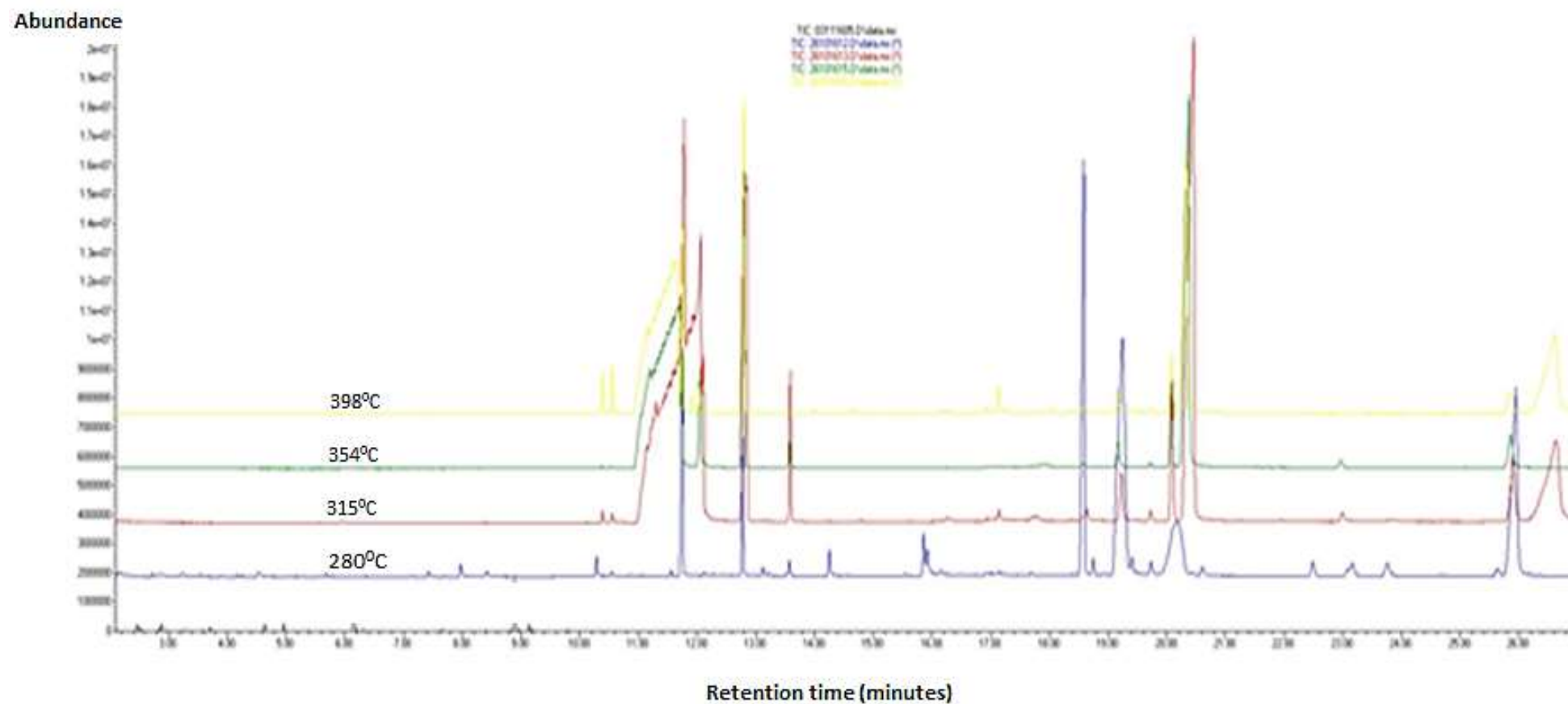


Figure 5.4 Overlay of chromatographic profiles of heroin at four different temperatures extracted in MeOH-DCM 50:50 at 354°C

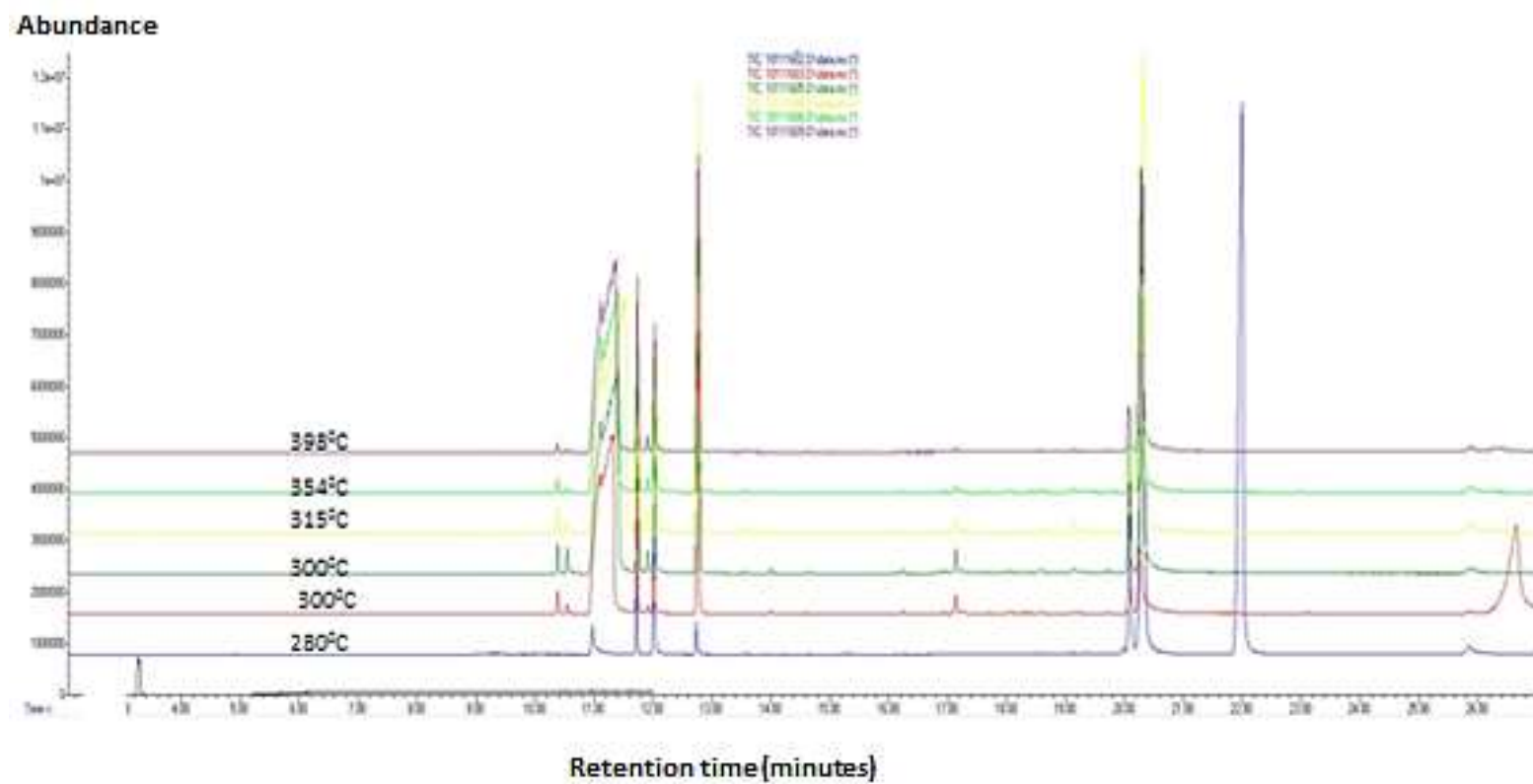


Figure 5.5 Overlay of chromatographic profiles of pyrolysis products of heroin at four different temperatures extracted in MeOH-DCM 90:10 at 354°C

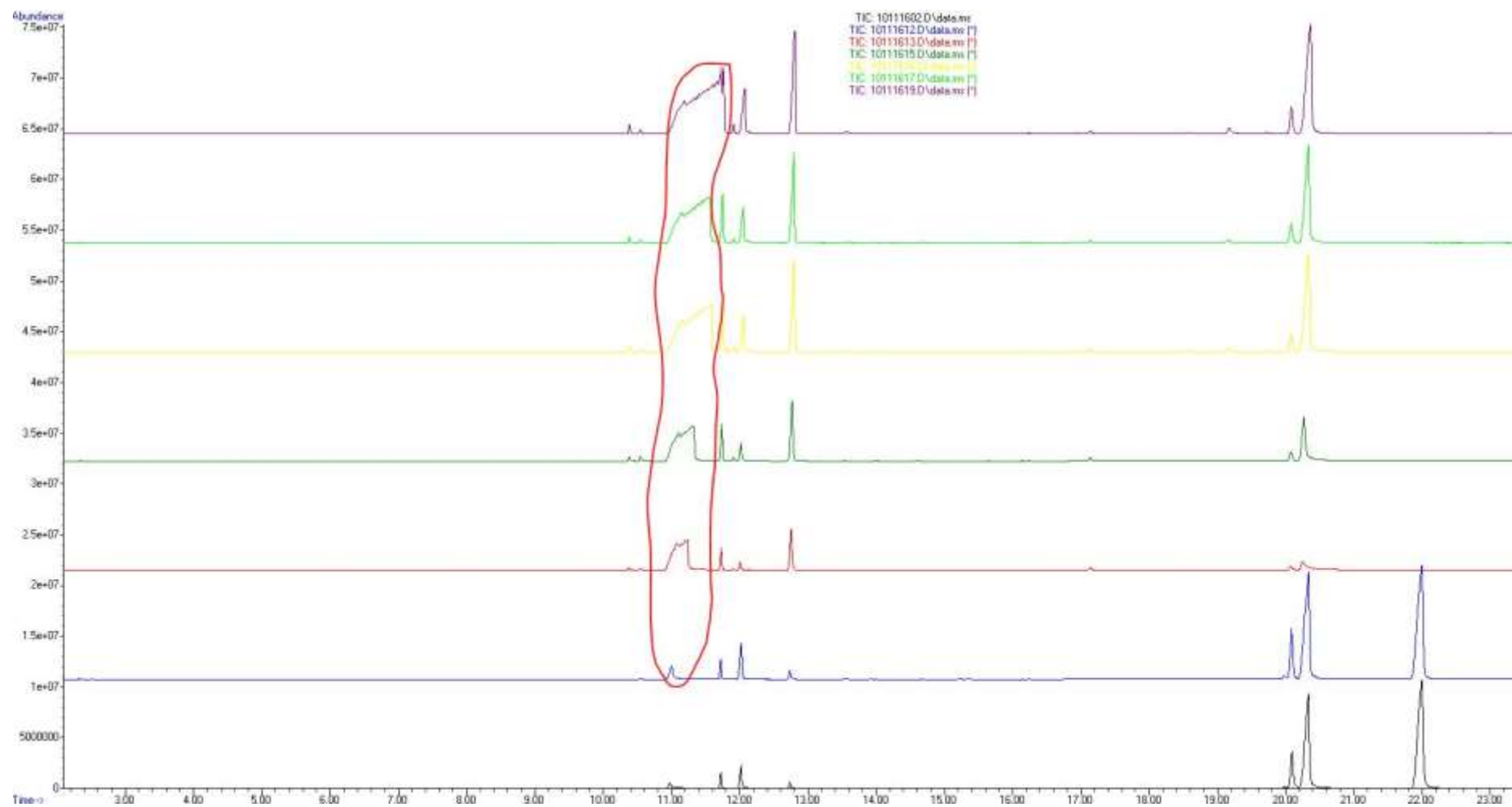


Figure 5.6 Overlay of chromatographic profiles of pyrolysis products of heroin extracted in MeOH-DCM 50:50 at 354°C, showing a large paracetamol peak at 11 minutes

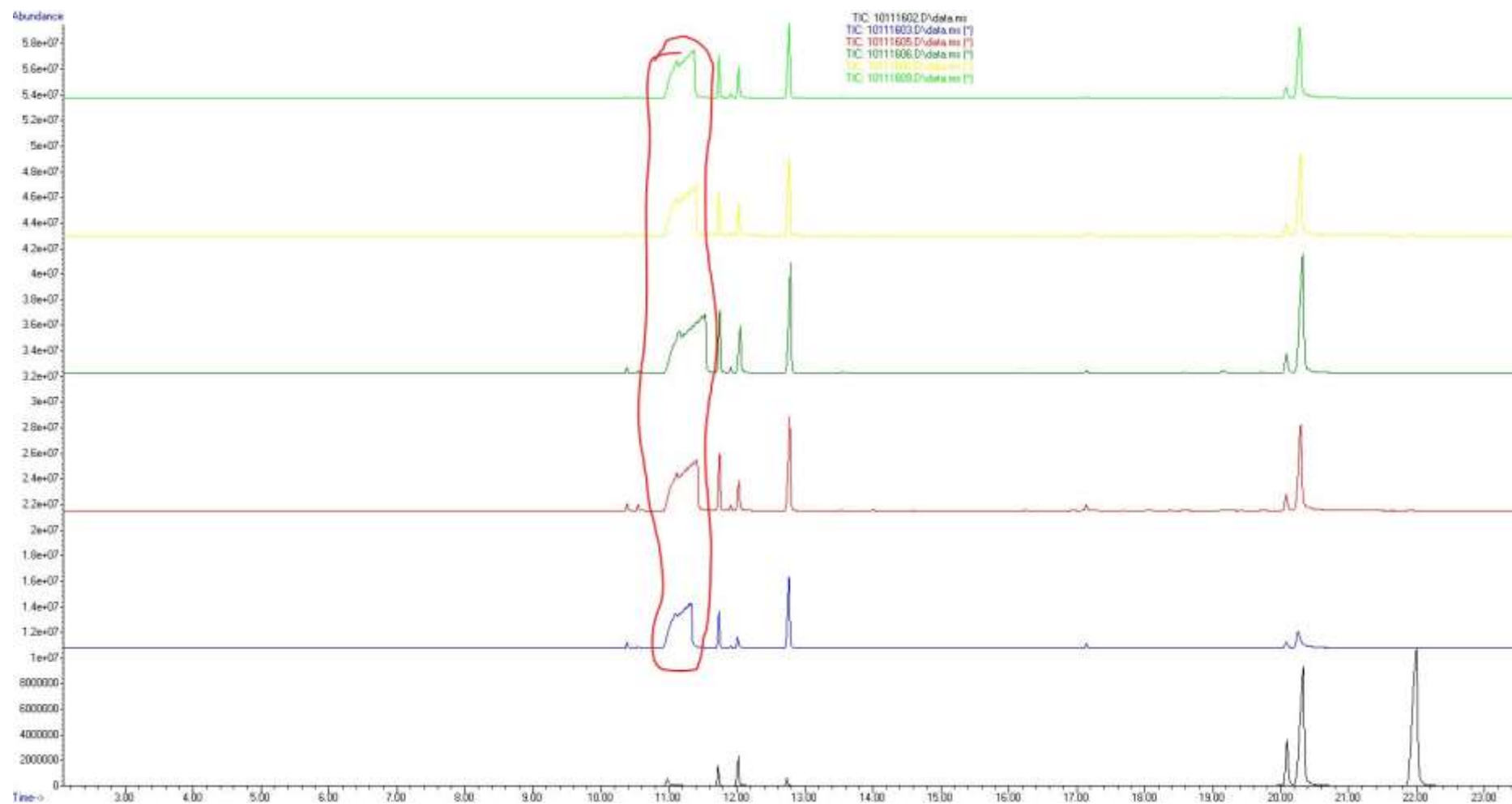


Figure 5.7 Overlay of chromatographic profile of pyrolysis products of heroin extracted in MeOH-DCM 90:10 at 354°C, also showing a large paracetamol peak at 11 minutes.

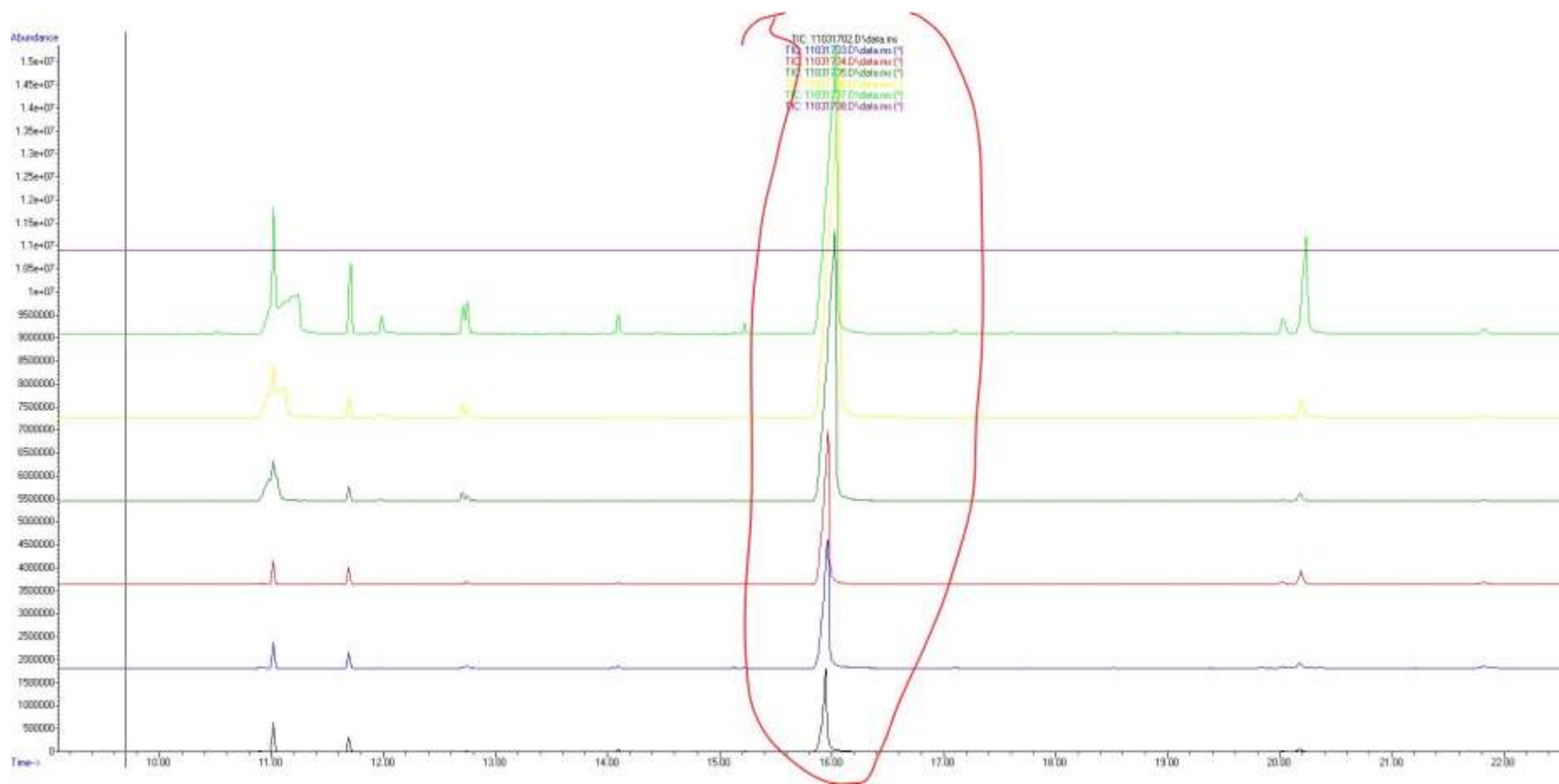
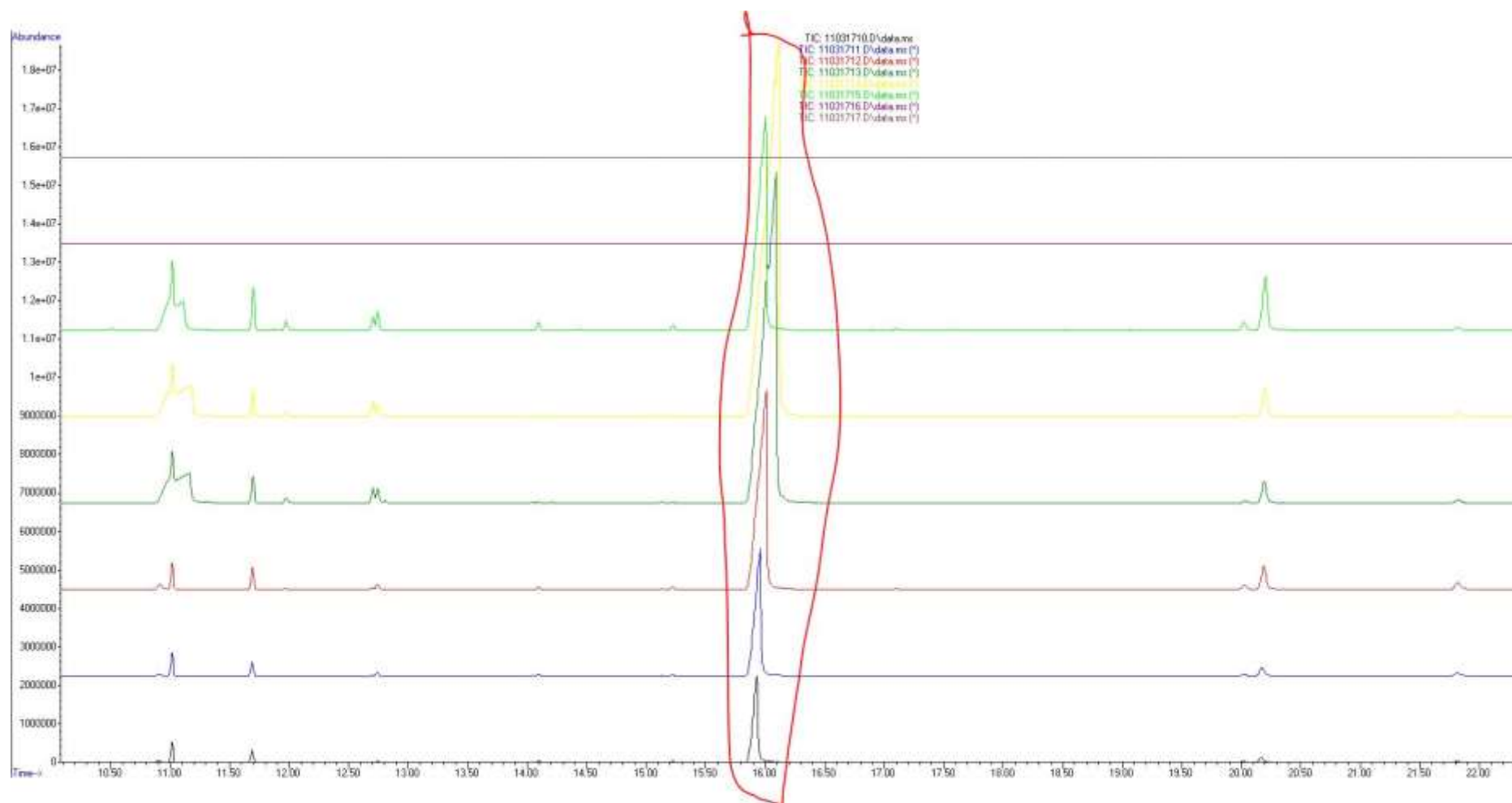


Figure 5.12 Overlay of chromatographic profile of pyrolysis products of EFV-heroin for within-can repeatability extracted in MeOH-DCM 50:50 at 354°C, showing EFV peak at 16 minutes



**Figure 5.13 Chromatographic profile of pyrolysis products of EFV-heroin for within-can repeatability extracted with MeOH-DCM 90:10, also showing EFV peak at 16 minutes**

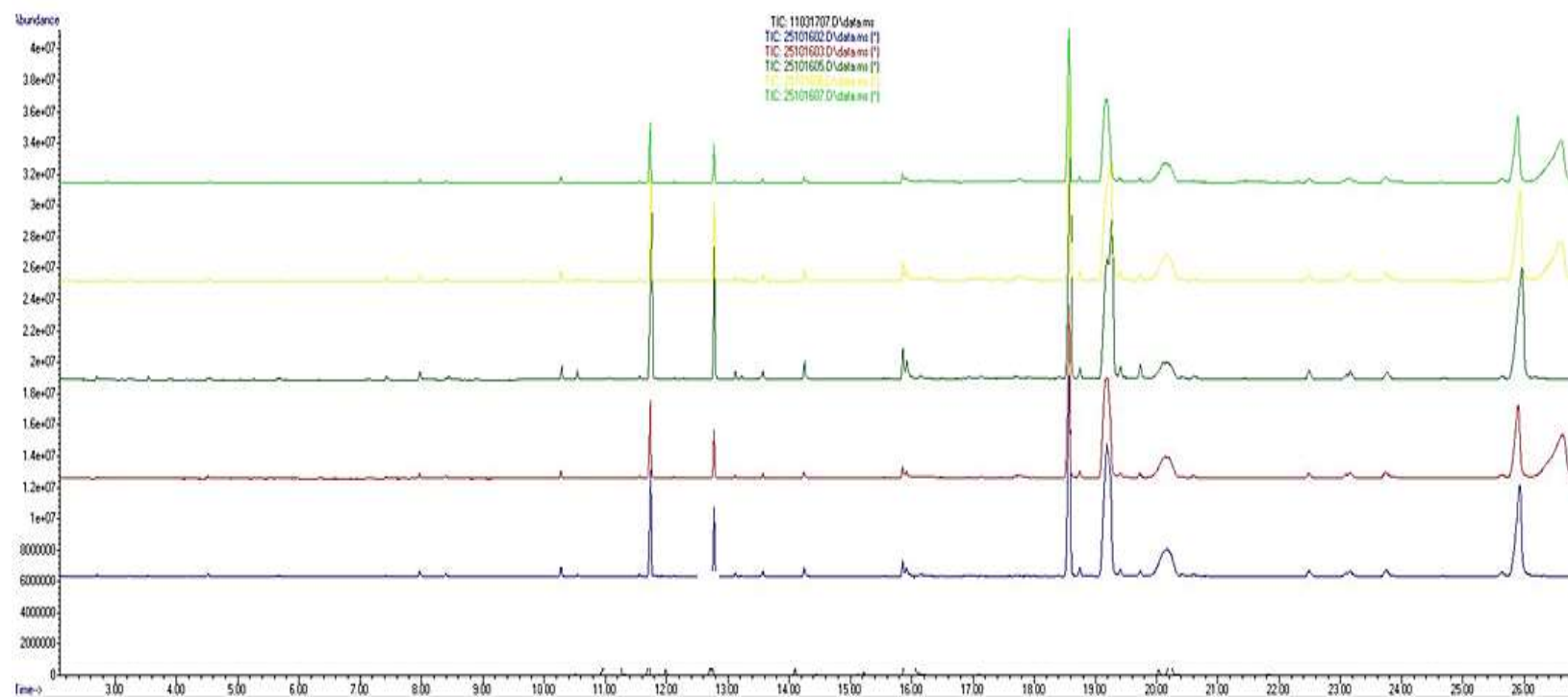


Figure 6.3 Overlay of chromatographic profiles for pyrolysis products of heroin extracted in MeOH-DCM 50:50 at 354<sup>0</sup>C



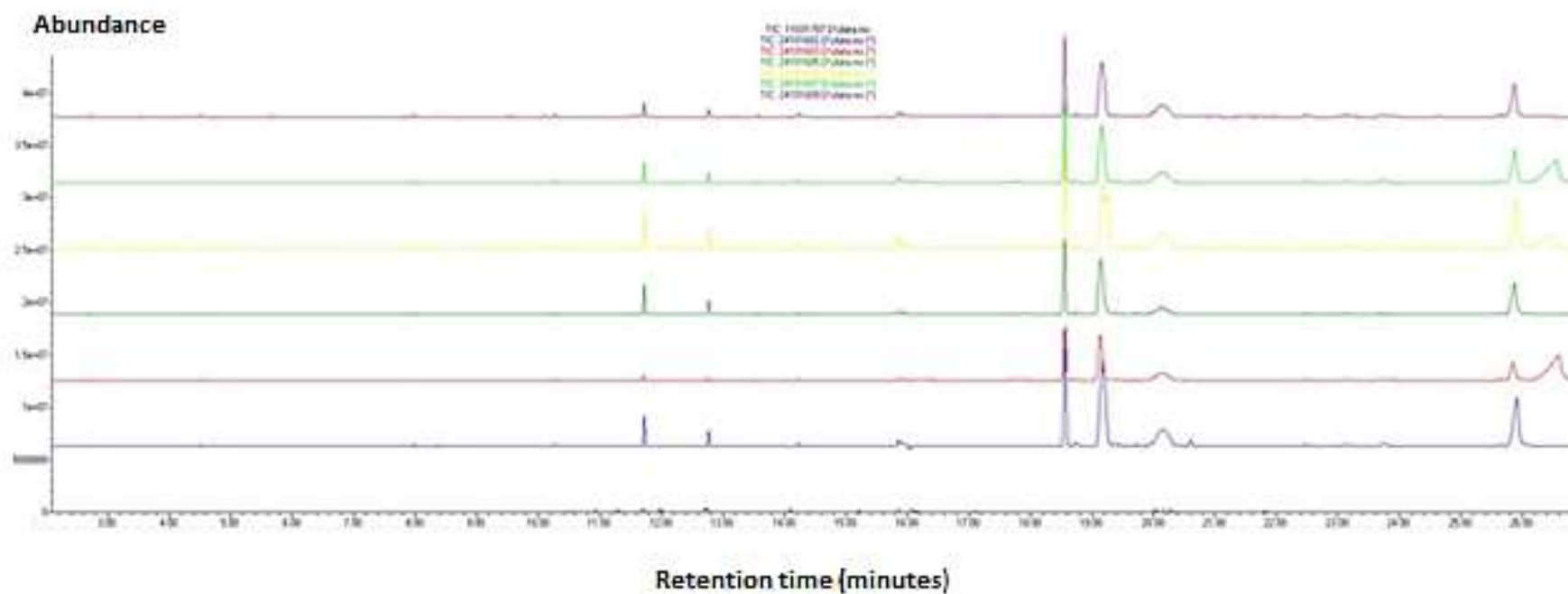


Figure 6.4 Overlay of chromatographic profiles of pyrolysis products of opium extracted in MeOH-DCM 90:10 at 354<sup>0</sup>C

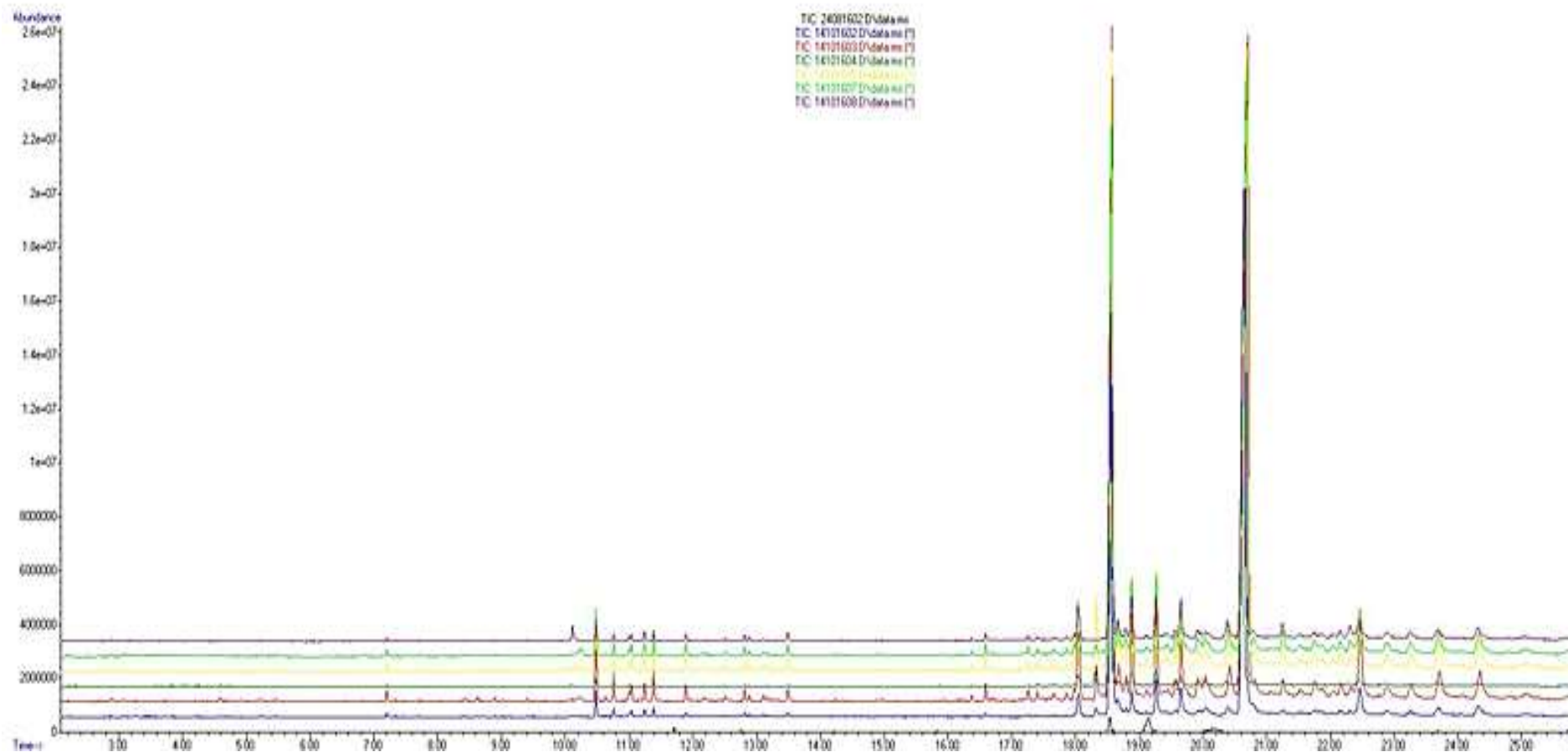


Figure 6.15 Overlay of chromatographic profiles of cannabis extracted in MeOH-DCM 50:50 at 354°C

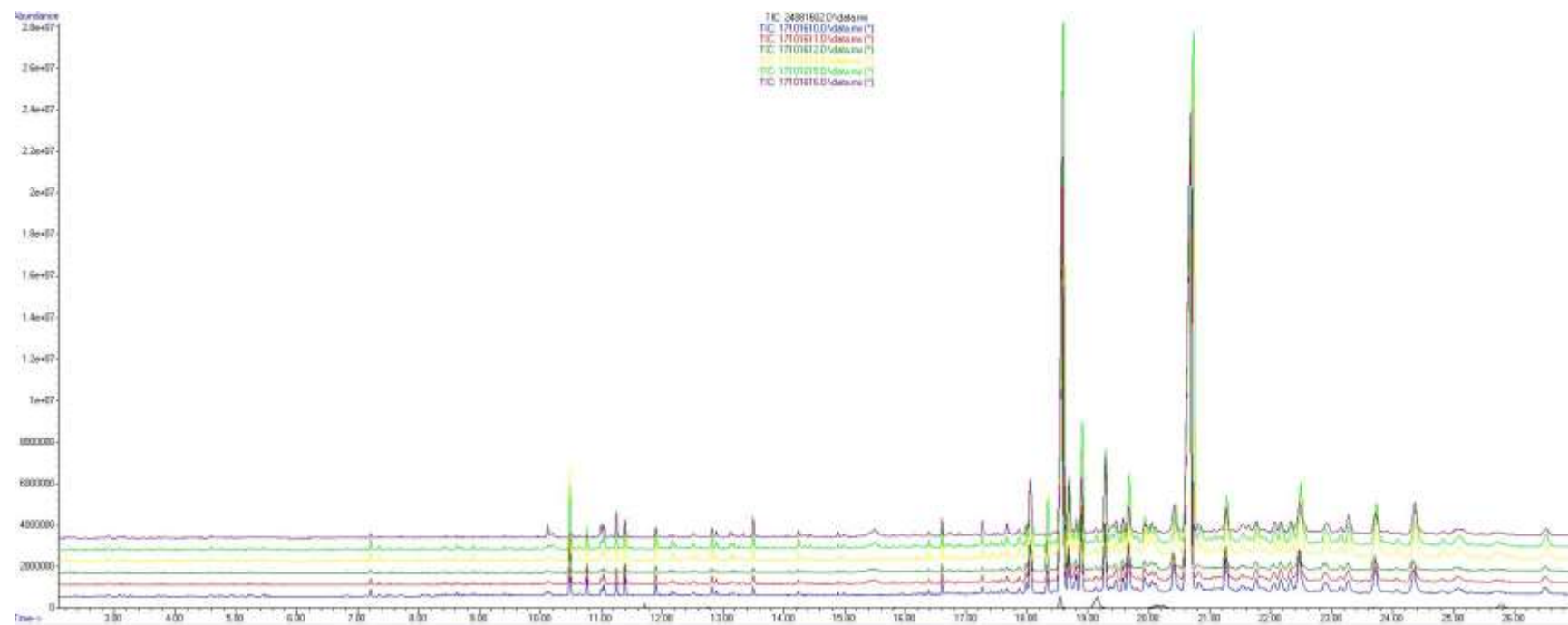


Figure 6.16 Overlay of chromatographic profiles of cannabis extracted in MeOH-DCM 90:10 at 354°C

**Appendix B. Statistical tables for pyrolysis products of different drugs (amphetamine, heroin, opium and cannabis) in single drugs and in mixtures with EFV**

**Table 4.26 %RSD results for amphetamine pyrolysis product vs peak area for within-can analytical repeatability extracted in MeOH-DCM 50:50 at 354<sup>0</sup>C**

Pyrolysis products	Pyrolysis product Abundance								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	RSD%
2-Furan carboxaldehyde-5 methyl	395422	383564	386745	391001	387676	ND	<b>388881.6</b>	<b>4516</b>	<b>1.16</b>
Benzaldehyde	1152712	1162610	1103211	1216546	1143242	1126716	<b>1150839</b>	<b>51568</b>	<b>4.48</b>
2-furan methanol	1249049	1234521	1227642	1211176	1222534	1106498	<b>1208570</b>	<b>762</b>	<b>0.06</b>
dimethyl acetal-benzaldehyde	80196	ND	80776	82152	81665	81315	<b>81220</b>	<b>4572</b>	<b>5.63</b>
alpha-methyl, Benzeneethanol	415229	ND	413721	421165	421334	411103	<b>416510</b>	<b>7368</b>	<b>1.77</b>
1-phenyl-1,2-propanedione ,	480531	ND	484261	497052	492226	480718	<b>486958</b>	<b>7368</b>	<b>1.51</b>
Isosorbide	1196787	1126671	ND	1224327	1115332	1243161	<b>1181256</b>	<b>503</b>	<b>0.04</b>
N-Formylamphetamine	93969	93421	94606	94365	93496	93511	<b>93895</b>	<b>2883</b>	<b>3.07</b>
N-acetylamphetamine	214832	214217	216327	212772	221002	214441	<b>215599</b>	<b>2632</b>	<b>1.22</b>

ND =not detected

**Table 4.27 %RSD results for amphetamine pyrolysis product vs peak area for analytical within-can repeatability extracted in MeOH-DCM 90:10 at 3540C**

Pyrolysis products	Pyrolysis product Abundance								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	RSD%
2-Furan carboxaldehyde-5 methyl	446920	465567	453633	445763	451102	446521	<b>451584.3</b>	<b>7499.689</b>	<b>1.66</b>
Benzaldehyde	332150	332510	332147	332151	332150	332155	<b>332210.5</b>	<b>146.75</b>	<b>0.04</b>
1-phenyl-2-propanol	331150	331153	331153	332101	331151	331151	<b>331309.8</b>	<b>387.59</b>	<b>0.12</b>
1-phenyl-1,2-propanedione	213172	213170	213210	213100	213111	213051	<b>213135.7</b>	<b>58.44</b>	<b>0.03</b>
N-Formylamphetamine	836003	836003	836401	836002	836006	836002	<b>836069.5</b>	<b>162.41</b>	<b>0.02</b>
N-acetylamphetamine	246951	246702	246977	244951	246954	246702	<b>246539.5</b>	<b>788.50</b>	<b>0.32</b>

**Table 4.28 %RSD results for amphetamine pyrolysis product vs peak area for inter-can analytical repeatability extracted in MeOH-DCM 50:50 at 354<sup>0</sup>C**

Pyrolysis product	Relative abundance (product peak area )								
	Can 1	Can 2	Can 3	Can 4	Can5	Can 6	Average	Stdev	%RSD
2-Furan carboxaldehyde-5 methyl	395422	2834342	89521	2267231	2480004	ND	1613304	1272289	78.86231
Benzaldehyde	1152712	5307573	202946	22780186	20218632	4169196	8971874	9916504	110.5288
2-furan methanol	1249049	17460378	25781	23385488	28153910	11064989	13556599	11538587	85.11417
dimethyl acetal-benzaldehyde	80196	ND	40595	5836196	3626315	340423	1984745	2628652	132.4428
alpha-methyl, Benzeneethanol	1152712	5307573	202946	22780186	20218632	4169196	8971874	9916504	110.5288
1-phenyl-1,2-propanedione ,	1249049	17460378	25781	23385488	28153910	11064989	13556599	11538587	85.11417
Isosorbide	1196787	6253603	ND	22153807	14370445	14619318	11718792	8138101	69.44488
N-Formylamphetamine	93969	434212	44609	786532	50007	401475	301800.7	295016.5	97.75212
N-acetylamphetamine	214832	522217	312027	561004	300043	350405	376754.7	135716.4	36.02249

ND = not detected

**Table 4.29 %RSD results for amphetamine pyrolysis products vs peak area for inter-can analytical repeatability extracted in MeOH-DCM 90:10 at 354°C**

Pyrolysis products	Pyrolysis product Abundance								
	can 1	can 2	can 3	can 4	can 5	can 6	Average	Stdev	RSD%
2-Furan carboxaldehyde-5 methyl	446920	578321	1932353	1229394	683311	3639779	<b>1418346.33</b>	<b>1218544.83</b>	<b>85.91</b>
Benzaldehyde	332150	192961	23555507	886210	327910	27682037	<b>8829462.5</b>	<b>13072422</b>	<b>148.05</b>
1-phenyl-2-propanol	331150	212238	6136364	2333520	823990	11274158	<b>3518570</b>	<b>4399058.38</b>	<b>125.02</b>
1-phenyl-1,2-propanedione	213172	143178	1633245	497467	293483	9357204	<b>2022958.16</b> <b>7</b>	<b>3635110.60</b>	<b>179.69</b>
N-Formylamphethamine	836003	1239531	17901073	7579575	4045002	59812121	<b>15235550.8</b> <b>3</b>	<b>22721151</b>	<b>149.13</b>
N-acetylamphetamine	246951	174358	2682964	932705	522124	357366	<b>819411.33</b>	<b>951980.49</b>	<b>116.18</b>

**Table 4.31 %RSD results for EFV-amphetamine pyrolysis product vs peak area for within-can analytical repeatability extracted in MeOH-DCM 50:50 at 354<sup>0</sup>C**

Pyrolysis product	Relative peak area across the six tin cans								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	RSD%
2-Furan carboxaldehyde-5 methyl	395422	383564	386745	391001	387676	ND	<b>388881.6</b>	<b>4515.64</b>	<b>1.16</b>
Benzaldehyde	1152712	1162610	1103211	1216546	1143242	1126716	<b>1150840</b>	<b>51567.86</b>	<b>4.48</b>
2-furan methanol	1249049	1234521	1227642	1211176	1222534	1106498	<b>1208570</b>	<b>761.80</b>	<b>0.06</b>
dimethyl acetal-benzaldehyde	80196	ND	80776	82152	81665	81315	<b>81220.8</b>	<b>4571.52</b>	<b>5.62</b>
alpha-methyl, Benzeneethanol	415229	ND	413721	421165	421334	411103	<b>416510.4</b>	<b>7368.15</b>	<b>1.77</b>
1-phenyl-1,2-propanedione ,	480531	ND	484261	497052	492226	480718	<b>486957.6</b>	<b>7368.15</b>	<b>1.51</b>
Isosorbide	1196787	1126671	ND	1224327	1115332	1243161	<b>1181256</b>	<b>502.64</b>	<b>0.04</b>
N-Formylamphetamine	93969	93421	94606	94365	93496	93511	<b>93894.7</b>	<b>2882.83</b>	<b>3.07</b>
N-acetylamphetamine	214832	214217	216327	212772	221002	214441	<b>215598.5</b>	<b>2631.65</b>	<b>1.22</b>
EFV pyrolysis product 1	735848	745574	734471	729875	732001	744520	<b>737048.2</b>	<b>5965.58</b>	<b>0.81</b>
EFV	395422	383564	386745	391001	387676	ND	<b>388881.6</b>	<b>4515.64</b>	<b>1.16</b>

Key: ND = not detected



**Table 4.32 %RSD results for EFV-amphetamine pyrolysis product vs peak area for within-can analytical repeatability extracted in MeOH-DCM 90:10 at 35°C**

Pyrolysis product	Relative peak area across the six tin cans								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stddev	RSD%
2-Furan carboxaldehyde-5 methyl	ND	ND	25356554	25552339	25344765	25566632	<b>25455072.5</b>	<b>120802.78</b>	<b>0.47</b>
Benzaldehyde	5931887	5893187	5893186	5893884	5893266	5891789	<b>5899533.17</b>	<b>15865.01</b>	<b>0.27</b>
2-furan methanol	9577887	9577886	9578790	9576532	9577654	9576643	<b>9577565.33</b>	<b>852.34</b>	<b>0.01</b>
dimethyl acetal-benzaldehyde	1249049	1249048	1239887	1249056	1246765	1238067	<b>1245312</b>	<b>5019.41</b>	<b>0.40</b>
alpha-methyl, Benzeneethanol	545963	545965	545946	545599	545607	545956	<b>545839.33</b>	<b>183.20</b>	<b>0.03</b>
1-phenyl-1,2-propanedione ,	705610	705598	705600	705776	704321	706550	<b>705575.83</b>	<b>716.37</b>	<b>0.10</b>
Isosorbide	5429163	5429100	5428956	5429064	5428967	5329066	<b>5412386</b>	<b>40818.37</b>	<b>0.75</b>
N-Formylamphetamine	88401	88423	88412	88489	88400	88422	<b>88424.5</b>	<b>33.10</b>	<b>0.04</b>
N-acetylamphetamine	154265	154265	154456	154332	154667	154767	<b>154458.67</b>	<b>214.26</b>	<b>0.14</b>
EFV pyrolysis product 1	1776931	1776653	1776865	1698866	1776524	1776543	<b>1763730.33</b>	<b>31777.34</b>	<b>1.80</b>
EFV	230732	243330	232225	237864	238768	245033	<b>237992</b>	<b>5738.54</b>	<b>2.41</b>

**Key: ND = not detected**

**Table 4.33 %RSD results for amphetamine pyrolysis products vs peak area for inter-can analytical repeatability extracted in MeOH-DCM 90:10 at 354°C**

Pyrolysis product	Relative peak area across the six tin cans								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	RSD%
2-Furan carboxaldehyde-5 methyl	ND	383564	25356554	25552339	778543	456632	<b>10505526.4</b>	<b>13647419.15</b>	<b>129.91</b>
Benzaldehyde	5931887	ND	13647704	15559469	14453908	166532	<b>8313493.5</b>	<b>7180767.09</b>	<b>86.37</b>
2-furan methanol	9577887	ND	121155	288353	550987	4352622	<b>2978200.8</b>	<b>4084551.69</b>	<b>137.15</b>
dimethyl acetal-benzaldehyde	1249049	ND	1162114	1590992	1347800	1776292	<b>1425249.4</b>	<b>253469.30</b>	<b>17.78</b>
alpha-methyl, Benzeneethanol	545963	ND	771392	1019154	6721132	9833422	<b>3778212.6</b>	<b>4255207.03</b>	<b>112.62</b>
1-phenyl-1,2-propanedione ,	705610	88693	834380	1027662	444611	459982	<b>593489.67</b>	<b>332840.37</b>	<b>56.08</b>
Isosorbide	5429163	ND	6580054	18120023	5642231	6549902	<b>8464274.6</b>	<b>5422739.61</b>	<b>64.07</b>
N-Formylamphethamine	88401	ND	173407	556388	432218	2223114	<b>694705.6</b>	<b>875131.60</b>	<b>125.97</b>
N-acetylamphetamine	154265	ND	346166	718409	443232	4562221	<b>1244858.6</b>	<b>1865589.08</b>	<b>149.86</b>
EFV pyrolysis product 1	1776931	ND	5881957	11932547	7225454	223087	<b>5407995.2</b>	<b>4641267.95</b>	<b>85.82</b>
EFV	230732	ND	272225	2956722	332122	1115023	<b>981364.8</b>	<b>1162728.01</b>	<b>118.48</b>

Key: ND = not detected

**Table 4.34 %RSD results for pyrolysis products of EFV-amphetamine for inter-can can analytical repeatability extracted in MeOH-DCM 50:50 at 354°C**

Pyrolysis product	Relative peak area across the six tin cans								
	can 1	can 2	can 3	can 4	can 5	can 6	Average	Stdev	RSD%
2-Furan carboxaldehyde-5 methyl	395422	2834342	89521	2267231	2480004	ND	<b>1613304</b>	<b>1272289</b>	<b>78.86</b>
Benzaldehyde	1152712	5307573	202946	22780186	20218632	4169196	<b>8971874</b>	<b>9916504</b>	<b>110.52</b>
2-furan methanol	1249049	17460378	25781	23385488	28153910	11064989	<b>13556599</b>	<b>11538587</b>	<b>85.11</b>
dimethyl acetal-benzaldehyde	80196	ND	40595	5836196	3626315	340423	<b>1984745</b>	<b>2628652</b>	<b>132.44</b>
alpha-methyl, Benzeneethanol	415229	ND	379099	2388305	2214219	643043	<b>1207979</b>	<b>1005029</b>	<b>83.20</b>
1-phenyl-1,2-propanedione ,	480531	ND	134261	2137052	2011194	580398	<b>1068687</b>	<b>933706.2</b>	<b>87.37</b>
Isosorbide	1196787	6253603	ND	22153807	14370445	14619318	<b>11718792</b>	<b>8138101</b>	<b>69.44</b>
N-Formylamphetamine	93969	434212	44609	786532	50007	401475	<b>301801</b>	<b>295017</b>	<b>97.75</b>
N-acetylamphetamine	214832	522217	312027	561004	300043	350405	<b>376754.7</b>	<b>135716.4</b>	<b>36.02</b>
product 1	735848	12787407	625617	34005027	13150736	3999327	<b>10883994</b>	<b>12640895</b>	<b>116.14</b>
EFV	211326	2241461	311057	14021370	9264184	355804	<b>4400867</b>	<b>5856796</b>	<b>133.08</b>

**Table 4.37 %RSD results for analytical within-can repeatability for pyrolysis products of amphetamine extracted in the mobile phase at 354<sup>0</sup>C**

Pyrolysis product	Pyrolysis product peak area at 354 <sup>0</sup> C								
	ACS 1	ACS2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
Benzaldehyde	166.353	165.504	165.335	165.352	165.994	165.665	<b>165.70</b>	<b>0.40</b>	<b>0.24</b>
Furan methanol	50.994	49.009	50. 944	49.998	51.014	50.221	<b>50.25</b>	<b>0.83</b>	<b>1.65</b>
P2P	14.109	14.271	14. 326	14.76	13.786	14.86	<b>14.36</b>	<b>0.45</b>	<b>3.14</b>
Dimethyl amphetamine	80.626	81.098	80.302	80.441	81.118	80.223	<b>80.63</b>	<b>0.39</b>	<b>0.49</b>

**Table 4.38 %RSD results for pyrolysis product of amphetamine for analytical inter-can repeatability extracted in the MeOH-DCM 90:10 at 35<sup>40</sup>C**

Pyrolysis product	Pyrolysis product peak area at 354 <sup>0</sup> C								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
Benzaldehyde	16.772	16.772	16.772	16.771	16.769	17.001	<b>16.771</b>	<b>0.0012</b>	<b>0.008</b>
Dimethylamphetamine	60.258	59.3334	60.336	61.1	61.002	60.44	<b>60.41</b>	<b>0.63</b>	<b>1.05</b>
P2P	10.669	10.212	10.656	10.668	10.663	10.664	<b>10.59</b>	<b>0.18</b>	<b>1.74</b>
furan methanol	19.285	19.287	19.285	19.289	19.287	19.287	<b>19.29</b>	<b>0.0015</b>	<b>0.008</b>

**Table 4.39 %RSD results for inter-can analytical repeatability for pyrolysis products of amphetamine extracted with the mobile phase at 354<sup>o</sup>C**

Pyrolysis product	Pyrolysis product peak area at 354 <sup>o</sup> C								
	Can 1	Can 2	Can 3	Can 4	Can 5	Can 6	Average	Stdev	%RSD
Benzaldehyde	166.353	46.068	154.003	127.99	57.981	156.223	<b>118.10</b>	<b>52.86</b>	<b>44.76</b>
Furan methanol	50.994	54.994	35.091	67.453	98.993	78.894	<b>64.40</b>	<b>22.55</b>	<b>35.02</b>
P2P	14.109	14.123	66.99	45.143	13.786	33.786	<b>31.32</b>	<b>21.77</b>	<b>69.49</b>
Dimethyl amphetamine	80.626	36.21	97.098	123.99	67.762	88.11	<b>82.30</b>	<b>29.43</b>	<b>35.75</b>

**Table 4.40 %RSD results for pyrolysis products of amphetamine for analytical inter-can repeatability extracted in the MeOH-DCM 90:10 at 354<sup>o</sup>C**

Pyrolysis product	Pyrolysis product peak area at 354 <sup>o</sup> C								
	Can 1	Can 2	Can 3	Can 4	Can 5	Can 6	Average	Stdev	%RSD
Benzaldehyde	16.772	431	174.223	88.997	16.77	210.22	<b>156.33</b>	<b>156.30</b>	<b>99.98</b>
Dimethylamphetamine	60.258	258	60.876	47.068	10.66	36.771	<b>78.94</b>	<b>89.66</b>	<b>113.58</b>
P2P	10.669	341.695	57.33	32.261	91.408	68.662	<b>100.34</b>	<b>258.33</b>	<b>257.46</b>
furan methanol	19.285	68.332	13.99	18.082	427.423	88.11	<b>105.87</b>	<b>160.48</b>	<b>151.58</b>

**Table 4.43 %RSD results for EFV-amphetamine pyrolysis products vs peak area for within-can analytical repeatability extracted in the mobile phase at 354°C**

Pyrolysis product	Pyrolysis product peak area at 354°C								
	ACS 1	ACS 2	ACS 3	ACS4	ACS 5	ACS 6	Average	Stdev	%RSD
Benzaldehyde	790.989	751.684	790.989	793.897	791.673	793.99	<b>785.54</b>	<b>16.64</b>	<b>2.12</b>
Furan methanol	94.342	94.341	94.299	93.998	94.212	89.567	<b>93.46</b>	<b>1.91</b>	<b>2.05</b>
EFV pyrolysis product 1	160.065	160.443	160.564	159.443	160.443	161.005	<b>160.33</b>	<b>0.53</b>	<b>0.33</b>
P2P	57.498	57.308	56.998	57.398	57.4	57.786	<b>57.40</b>	<b>0.26</b>	<b>0.45</b>
Dimethyl amphetamine	5.537	5.537	5.334	5.675	5.347	5.475	<b>5.48</b>	<b>0.13</b>	<b>2.36</b>
EFV	130.956	130.967	129.776	131.09	131	131.221	<b>130.83</b>	<b>0.53</b>	<b>0.40</b>

**Table 4.44 %RSD results for EFV-amphetamine pyrolysis product vs peak area for within-can analytical repeatability extracted in MeOH-DCM 90:10 at 354°C**

Pyrolysis product	Pyrolysis product peak area at 354°C								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
Benzaldehyde	232.351	232.349	232.35	232.34	232.349	232.339	<b>232.35</b>	<b>0.005</b>	<b>0.0023</b>
Furan methanol	431.879	433.841	431.708	430.889	431.098	439.898	<b>433.22</b>	<b>3.44</b>	<b>0.79</b>
EFV pyrolysis product 1	510.065	510	513.32	510.335	513.998	510.332	<b>511.34</b>	<b>1.81</b>	<b>0.35</b>
P2P	325.67	323.672	323.656	323.157	323.673	323.6	<b>323.90</b>	<b>0.89</b>	<b>0.27</b>
Dimethyl amphetamine	22.711	22.463	23.767	22.221	23.274	23.756	<b>23.03</b>	<b>0.66</b>	<b>2.884</b>
EFV	335.042	334.987	334.092	333.989	335	335.876	<b>334.79</b>	<b>0.69</b>	<b>0.21</b>

**Table 4.45 %RSD results for EFV-amphetamine pyrolysis products vs peak area for within-can analytical repeatability extracted in the mobile phase at 354°C**

Pyrolysis product	Pyrolysis product peak area at 354°C								
	Can 1	Can 2	Can 3	Can 4	Can 5	Can 6	Average	Stdev	%RSD
Furan methanol	790.989	751.684	309.875	1036.91	118.088	880.42	<b>647.99</b>	<b>355.42</b>	<b>54.85</b>
Benzaldehyde	94.342	21.547	25.407	266.766	334.581	22.8	<b>127.57</b>	<b>138.56</b>	<b>108.61</b>
EFV pyrolysis product 1	160.065	74.972	85.735	525.159	585.134	127.789	<b>259.81</b>	<b>231.56</b>	<b>89.13</b>
P2P	57.498	29.672	14. 326	85.946	69.0944	87.053	<b>65.85</b>	<b>23.68</b>	<b>35.96</b>
Dimethyl amphetamine	5.537	ND	5.702	ND	ND	5.501	<b>5.58</b>	<b>0.11</b>	<b>1.92</b>
EFV	130.956	78.96	16.566	29.521	321.602	20.078	<b>99.61</b>	<b>117.32</b>	<b>117.78</b>



**Table 4.46 %RSD results for EFV-amphetamine pyrolysis product vs peak area for inter-can analytical repeatability extracted in MeOH-DCM 90:10 at 3540C**

Pyrolysis product	Pyrolysis product peak area at 354 <sup>0</sup> C								
	Can 1	Can 2	Can 3	Can 4	Can 5	Can 6	Average	Stdev	%RSD
Furan methanol	232.351	181.058	492.542	106.181	244.324	455	<b>285.24</b>	<b>154.39</b>	<b>54.137</b>
Benzaldehyde	431.879	407.817	301.017	243.583	928.366	125.882	<b>406.42</b>	<b>279.07</b>	<b>68.67</b>
EFV pyrolysis product 1	510.065	361.255	956.545	526.717	951.3811	325.099	<b>605.17</b>	<b>281.59</b>	<b>46.53</b>
P2P	325.67	220.037	740.277	157.025	327.288	98.021	<b>311.39</b>	<b>228.95</b>	<b>73.53</b>
Dimethyl amphetamine	22.711	15.735	44.915	10.919	23.274	34.022	<b>25.26</b>	<b>12.41</b>	<b>49.12</b>
EFV	335.042	206.116	1299.377	112.498	325.118	20.078	<b>383.04</b>	<b>465.10</b>	<b>121.42</b>

**Table 5.2 %RDS results for pyrolysis products of heroin vs peak area for within-can analytical repeatability extracted in MeOH-DCM 50:50 at 354<sup>0</sup>C**

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in each ACS in tin can 1								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
acetaminophen	212887618	211889422	212888774	212887661	212891170	218879551	<b>213720699.3</b>	<b>2558731.06</b>	<b>1.20</b>
meconin lactone	31461096	31461087	31461096	31687331	31452733	32140011	<b>31610559</b>	<b>275006.42</b>	<b>0.87</b>
hydrocortanine	1952080	1952080	1952088	1952082	1952080	1952911	<b>1952220.167</b>	<b>338.45</b>	<b>0.02</b>
diacetamate	12094103	12094103	12094413	12094103	12092321	12908891	<b>12229655.67</b>	<b>332756.85</b>	<b>2.72</b>
caffeine	120994103	120994103	120994100	120897811	120994103	120994102	<b>120978053.7</b>	<b>39310.72</b>	<b>0.03</b>
acetylcodeine	11097064	11097054	11097064	11097062	11097000	11180466	<b>11110951.67</b>	<b>34054.94</b>	<b>0.31</b>
6-monoacetylmorphine	59449638	59449666	59355225	59488892	58877745	59449044	<b>59345035</b>	<b>233180.58</b>	<b>0.39</b>
papaverine	345543828	345543828	345554376	352334561	345571291	355567812	<b>348352616</b>	<b>4455545.65</b>	<b>1.28</b>

**Table 5.3 %RSD results for heroin pyrolysis products vs peak area for within can analytical repeatability extracted in MeOH-DCM 9010 at 354<sup>0</sup>C.**

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in each ACS in tin can 1								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
acetaminophen	320331996	320331996	320398790	320059678	320033189	320331996	<b>320247940.8</b>	<b>158437.70</b>	<b>0.05</b>
meconin lactone	111381498	111386758	111381498	111381498	111381498	111381498	<b>111382374.7</b>	<b>2147.39</b>	<b>0.002</b>
hydrocortanine	21219928	20323428	21219944	21219927	21222298	21219928	<b>21070908.83</b>	<b>366190.55</b>	<b>1.74</b>
caffeine	239598362	239587601	229522100	239582100	239598378	241576382	<b>238244153.8</b>	<b>4346049.26</b>	<b>1.82</b>
morphine	28461545	27659908	28461500	28461544	28461545	28461545	<b>28327931.17</b>	<b>327263.18</b>	<b>1.15</b>
acetylcodeine	44995255	46145335	44995255	44995255	44995255	44995255	<b>45186935</b>	<b>469518.19</b>	<b>1.04</b>
6-onoacetylmorphine	362335492	362335495	362335889	362335394	360443667	362335412	<b>362020224.8</b>	<b>772352.47</b>	<b>0.21</b>
papaverine	35240948	35240943	35240679	35240950	35248776	35248876	<b>35243528.67</b>	<b>4104.73</b>	<b>0.01</b>

**Table 5.4 %RSD results for heroin pyrolysis products vs peak area for inter-can analytical repeatability extracted in MeOH-DCM 50:50 at 354°C.**

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in each ACS in tin can 1								
	Can 1	Can 2	Can 3	Can 4	Can 5	Can 6	Average	Stddev	%RSD
acetaminophen	257004	29950818	10193917	20644053	205283249	56493507	<b>53803758</b>	<b>76669254</b>	<b>142.50</b>
Meconin lactone	31461096	176760229	2431104811	613399068	31213203	31213203	<b>552525268</b>	<b>947523772.1</b>	<b>171.49</b>
hydrocortanine	1952080	119845872	1952088	87580571	93546169	33381192	<b>56376329</b>	<b>50668447</b>	<b>89.86</b>
diacetamate	12094103	12094103	12094413	12094103	12092321	12908891	<b>12229656</b>	<b>332756.8</b>	<b>2.72</b>
caffeine	120994103	302775207	223510540	2406558813	136684063	2334369	<b>532142849</b>	<b>923842691.3</b>	<b>173.61</b>
acetylcodeine	11097064	748449657	53185688	52028045	26165585	545693086	<b>239436521</b>	<b>322590193.8</b>	<b>134.73</b>
6-monoacetylmorphine	59449638	594221121	450603521	451376933	181251264	640535944	<b>396239737</b>	<b>230008874.1</b>	<b>58.05</b>
papaverine	44071165	53803758	28069109	25299060	-	33034605	<b>15334036</b>	<b>39308558</b>	<b>256.35</b>

**Table 5.5 %RSD results for heroin pyrolysis product vs peak area for inter-can analytical repeatability extracted in MeOH-DCM 90:10 at 354<sup>0</sup>C.**

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in each ACS in tin can 1								
	Can 1	Can 2	Can 3	Can 4	Can 5	Can 6	Average	Stdev	%RSD
acetaminophen	320331996	396000790	3410253	264865981	1338855779	117763201	<b>406871333.3</b>	<b>478065290.1</b>	<b>117.50</b>
meconin lactone	111381498	857316179	167520732	140537964	665683830	63496562	<b>334322794.2</b>	<b>338155992</b>	<b>101.15</b>
hydrocortanine	21219928	92202163	-	18283926	570508385	79879780	<b>156418836.4</b>	<b>233886810.7</b>	<b>149.53</b>
caffeine	239598362	350815815	93975996	302370046	179960801	120994102	<b>214619187</b>	<b>101385732.4</b>	<b>47.24</b>
morphine	28461545	40857791	677419881	28087078	-	29198804	<b>193890888.5</b>	<b>322404405.4</b>	<b>166.28</b>
acetylcodeine	44995255	90693265	268339550	57161497	146734286	80583010	<b>114751143.8</b>	<b>83115599.31</b>	<b>72.43</b>
6-onoacetylmorphine	362335492	801873320	11505674	400843784	1178798151	716846446	<b>578700477.8</b>	<b>407225834</b>	<b>70.37</b>
papaverine	35240948	65583531	440600161	41368198	109845884	9770758	<b>117068246.7</b>	<b>162058442.1</b>	<b>138.43</b>

**Table 5.7 %RSD results for EFV-heroin pyrolysis product vs peak area for within-can analytical repeatability extracted in MeOH-DCM 50:50 at 354<sup>0</sup>C**

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in each ACS in tin can 1								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	stdev	%RSD
acetaminophen	257004	257004	257006	248056	257002	257003	<b>255512.5</b>	<b>3652.92</b>	<b>1.43</b>
benzaldehyde	9232963	9232963	9232958	9232963	9357567	9232963	<b>9253730</b>	<b>50869.78</b>	<b>0.55</b>
Meconin lactone	5067986	5344298	5135628	5026607	5065631	5067016	<b>5117861</b>	<b>116376.44</b>	<b>2.27</b>
caffeine	104841	103983	104841	104799	104563	105100	<b>1047138</b>	<b>425.10</b>	<b>0.41</b>
hydrocortanine	258783	258783	257779	257954	257691	257639	<b>2581058</b>	<b>536.10</b>	<b>0.21</b>
EFV	50854846	58600434	58854846	58600422	58022876	58056361	<b>57164964</b>	<b>3108858.50</b>	<b>5.44</b>
6-monoacetylmorphine	1534479	1537663	1534471	1534761	1538611	1534775	<b>1535793</b>	<b>1844.65</b>	<b>0.12</b>
diacetylmorphine	146452	146236	147562	146443	146531	146725	<b>146658</b>	<b>470.02</b>	<b>0.32</b>

**Table 5.8 %RSD results for EFV-heroin pyrolysis products vs peak area for within-can analytical repeatability extracted in MeOH-DCM 90:10**

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in each ACS in tin can 1								
	ACS1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
EFV 1	1729.197	1743	1576.1	1665.99	1755.77	1765.88	<b>1756.88</b>	<b>72.725</b>	<b>4.14</b>
Meconin	307.21	308.33	309.3	307.33	307.77	307.211	<b>307.86</b>	<b>0.83</b>	<b>0.27</b>
morphine	1017.27	1011.56	1119.11	1034.44	1045.45	1030.193	<b>1043.00</b>	<b>39.21</b>	<b>3.76</b>
Acetyl codeine	847.513	884.33	887.462	803.345	863.22	864.33	<b>858.37</b>	<b>30.74</b>	<b>3.58</b>
3,6 Acetyl morphine	303.773	304.33	303.773	304.66	321.55	-	<b>307.62</b>	<b>7.79</b>	<b>2.53</b>
EFV	49133700	49356002	49876338	48443521	49887651	49946532	<b>49440624</b>	<b>590118.69</b>	<b>1.19</b>

**Table 5.9 %RSD results for EFV-heroin pyrolysis products vs peak area for inter-can analytical repeatability extracted in MeOH-DCM 50:50 at 354°C**

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in each ACS in tin can 1								
	Can 1	Can 2	Can 3	Can 4	Can 5	Can 6	Average	stdev	%RSD
acetaminophen	257004	396834	659277	880930	16490886	91734524	<b>18403243</b>	<b>36487067</b>	<b>198.26</b>
Meconin lactone	5067986	5344298	7712336	4926207	7086775	26576106	<b>9452285</b>	<b>8466774</b>	<b>89.57</b>
diacetamate	-	29724	62566	-	-	73666987	<b>24586426</b>	<b>42505016</b>	<b>172.88</b>
caffeine	104841	132475	186627	2381009	4462901	10073830	<b>2890281</b>	<b>3924533</b>	<b>135.78</b>
hydrocortanine	258783	57927	1022939	1385508	3166828	11510801	<b>2900464</b>	<b>4360907</b>	<b>150.35</b>
EFV	50854846	99720746	120145815	321691767	517619611	379987608	<b>2483367322</b>	<b>185873560</b>	<b>74.85</b>
acetylcodeine	-	666061	1617674	333916	1043863	9095661	<b>2551435</b>	<b>3689295</b>	<b>144.60</b>
6-monoacetylmorphine	1534479	2788130	8148083	4255538	10857550	69154242	<b>16123004</b>	<b>26211132</b>	<b>162.57</b>
diacetylmorphine	257004	396834	659277	880930	16490886	91734524	<b>18403243</b>	<b>36487067</b>	<b>198.26</b>



**Table 5.10 %RSD results for EFV-heroin pyrolysis product vs peak area for inter-can analytical repeatability extracted in MeOH-DCM 90:10 MeOH-DCM at 354<sup>0</sup>C**

Pyrolysis product	Peak area								
	Can1	Can 2	Can 3	Can 4	Can 5	Can 6	Average	Stdev	%RSD
Meconin lactone	-	8825105	11117023	10916046	17770172	4873576	<b>9821548.2</b>	<b>4708310.9</b>	<b>47.94</b>
caffeine	141382	428636	6466801	6456905	5912119	129096	<b>3255823.2</b>	<b>3319107.3</b>	<b>101.94</b>
hydrocatamine	946652	2242164	5956857	5186789	7650047	448622	<b>3738521.8</b>	<b>2938556.9</b>	<b>78.60</b>
EFV	118453632	252498770	318800804	789251802	277530003	69670715	<b>304367621</b>	<b>256272617</b>	<b>84.20</b>
Acetyl codeine	1068648	2838179	1453417	1633813	5342653	535700	<b>2145401.7</b>	<b>1743184.5</b>	<b>81.25</b>
6-monocaetyl morphine	6002978	16463693	15422431	19681060	40100849	3507949	<b>16863160</b>	<b>13011312</b>	<b>77.16</b>
diacetylmorphine	1933873	5878396	2384003	2603276	2056635	423593	<b>2546629.3</b>	<b>1802951.7</b>	<b>70.80</b>

**Table 5.13 %RSD results for heroin pyrolysis product vs peak area for within-can analytical repeatability extracted in the mobile phase at 354°C.**

Pyrolysis product	Peak area								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
Morphine	741.359	742.4	741.359	741.39	741.564	742.017	741.68	0.45	0.06
Meconin	133.462	133	134	133.78	133.433	133.422	133.48	0.32	0.24
Papaverine	27.513	28.955	27.56	27.563	27.434	28	27.763	0.64	2.32

**Table 5.14 %RSD results for heroin pyrolysis products vs peak area for within-can analytical repeatability extracted in MeOH-DCM 90:10 at 354°C.**

Pyrolysis product	Peak area								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	<b>Average</b>	<b>Stdev</b>	<b>%RSD</b>
Morphine	20.411	21.3	20.4	20.202	20.111	20.006	<b>20.405</b>	<b>0.43</b>	<b>2.09</b>
Meconin	19.914	19.866	19.891	19.899	19.985	19.899	<b>19.9126</b>	<b>0.04</b>	<b>0.20</b>
Noscapine	51.108	50.998	51.839	51.607	51.009	50.321	<b>51.147</b>	<b>0.56</b>	<b>0.95</b>
Papaverine	27.019	27.001	27	26.978	27.323	27.003	<b>27.054</b>	<b>0.12</b>	<b>0.45</b>

**Table 5.15 %RSD results for heroin pyrolysis products of heroin for in-between can repeatability extracted in the mobile phase at 354<sup>0</sup>C**

Pyrolysis product	Peak area								
	Can 1	Can 2	Can 3	Can 4	Can 5	Can 6	Average	Stdev	%RSD
Morphine	741.359	8732.52	8021.034	181.168	6009	9754	5573.18	4033.85	72.38
Meconin	133.462	3334.58	3334.587	168.475	1688.395	1229.82	1648.227	1592.04	96.59
Noscapine	-	3956.28	3761.615	-	2660.576	9680.84	5014.83	698.69	13.93
Papaverine	27.513	1573.28	1573.281	-	1758.039	1479	1282.22	808.38	63.05

**Table 5.16 %RSD results for heroin pyrolysis products vs peak area for inter-can analytical repeatability test extracted in the MeOH-DCM 90:10 at 354<sup>0</sup>C**

Pyrolysis product	Peak area								
	Can1	Can 2	Can 3	Can 4	Can 5	Can 6	Average	Stdev	%RSD
Morphine	20.411	204.394	77.54	12.03	12.789	116.175	<b>73.89</b>	<b>69.82</b>	<b>94.49</b>
Meconin	19.914	116.044	58.091	14.89	15.504	47.9	<b>1648.23</b>	<b>1592.04</b>	<b>96.59</b>
Noscapine	51.108	-	170.39	17.88	-	-	<b>5014.83</b>	<b>698.69</b>	<b>13.938</b>
Papaverine	27.019	-	43.281	-	15.669	-	<b>1282.22</b>	<b>808.38</b>	<b>63.05</b>

**Table 5.19 %RSD results for pyrolysis products of EFV-heroin for within-can analytical repeatability test extracted in the mobile phase at 354<sup>0</sup>C**

Pyrolysis product	Peak area								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
EFV 1	1596.38	1583.7	1573.1	1577.9	1557.7	1577.1	<b>1577.65</b>	<b>11.59</b>	<b>0.73</b>
Meconin	307.312	307.565	307.656	307.56	307.211	307.211	<b>307.42</b>	<b>0.18</b>	<b>0.06</b>
morphine	76.3	76.3	76.322	76.311	76.355	76.311	<b>76.32</b>	<b>0.02</b>	<b>0.02</b>
Acetyl codeine	847.513	847.333	857.241	847.55	847.511	847.321	<b>849.08</b>	<b>3.65</b>	<b>0.43</b>
EFV	49133700	49133700	49133711	49145561	49133722	49145531	<b>49137654.17</b>	<b>5580.38</b>	<b>0.01</b>

**Table 5.20 %RSD results for pyrolysis products of within-can analytical repeatability test extracted in the MeOH-DCM 90:10 at 354<sup>0</sup>C**

Pyrolysis product	Peak area								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
EFV 1	1729.197	1743	1576.1	1665.99	1755.77	1765.88	<b>1756.88</b>	<b>72.725</b>	<b>4.14</b>
Meconin	307.21	308.33	309.3	307.33	307.77	307.211	<b>307.86</b>	<b>0.83</b>	<b>0.27</b>
morphine	1017.27	1011.56	1119.11	1034.44	1045.45	1030.193	<b>1043.00</b>	<b>39.21</b>	<b>3.76</b>
Acetyl codeine	847.513	884.33	887.462	803.345	863.22	864.33	<b>858.37</b>	<b>30.74</b>	<b>3.58</b>
3,6 Acetyl morphine	303.773	304.33	303.773	304.66	321.55	-	<b>307.62</b>	<b>7.79</b>	<b>2.53</b>
EFV	49133700	49356002	49876338	48443521	49887651	49946532	<b>49440624</b>	<b>590118.69</b>	<b>1.19</b>

**Table 5.21 %RSD results for pyrolysis products of EFV-heroin for in-between can analytical repeatability test extracted in the mobile phase at 354<sup>0</sup>C**

Pyrolysis product	Peak area								
	Can1	Can 2	Can 3	Can 4	Can 5	Can 6	Average	Stdev	%RSD
EFV 1	1596.38	5807.77	453.141	3818	438.254	435.141	<b>2091.45</b>	<b>2244.89</b>	<b>107.34</b>
Meconin	307.312	559.886	148.171	61.843	9754.14	148.171	<b>1829.92</b>	<b>3886.06</b>	<b>212.36</b>
morphine	76.300	130.193	502.57	559.886	1229.817	1153.439	<b>608.71</b>	<b>491.65</b>	<b>80.77</b>
Acetyl codeine	847.513	-	-	1634.599	327.551	9860.824	<b>3167.62</b>	<b>4494.37</b>	<b>141.88</b>
EFV	49133700	983.344	847.623	1132810	6242900	12221.64	<b>9420577</b>	<b>19605581</b>	<b>208.11</b>

**Table 5.22 %RSD results for pyrolysis products of EFV-heroin for in-between can analytical repeatability test extracted in the MeOH-DCM 90:10 at 354<sup>0</sup>C**

Pyrolysis product	Peak area								
	Can1	Can 2	Can 3	Can 4	Can 5	Can 6	Average	Stdev	%RSD
EFV 1	1729.197	169393	438.254	185873	169391	1577.100	<b>88066.93</b>	<b>95296.42</b>	<b>108.21</b>
Meconin	307.215	988.703	130.193	988.703	678.331	307.211	<b>566.73</b>	<b>372.52</b>	<b>65.73</b>
morphine	1017.278	863.233	76.322	286.577	76.355	130.193	<b>408.33</b>	<b>421.99</b>	<b>103.35</b>
Acetyl codeine	847.513	220.881	465.462	803.345	-	202.282	<b>507.90</b>	<b>308.31</b>	<b>60.70</b>
3,6 Acetyl morphine	303.773	184.849	303.773	6624.612	453.776	-	<b>1574.16</b>	<b>2824.90</b>	<b>179.46</b>
EFV	49133700	39576000	38609800	98332000	3.5E+08	3000528	<b>96511788</b>	<b>128105735</b>	<b>132.74</b>

**Table 6.2 %RSD results for pyrolysis products of heroin for within-can repeatability extracted in MeOH-DCM 50:50 at 354<sup>0</sup>C**

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in tin can 1								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
meconin	62124853	61999073	62053296	62300876	61000547	61667443	<b>61857681</b>	<b>468386.34</b>	<b>0.76</b>
hydrocortanine	27829506	27829506	27650058	27847786	26888567	28475460	<b>27753481</b>	<b>509995.93</b>	<b>1.84</b>
codeine	190659502	190659200	190659502	18900768	189576883	187563322	<b>189686530</b>	<b>1251378.6</b>	<b>0.66</b>
morphine	288507145	288507145	288600781	288654903	299435221	288511679	<b>290369479</b>	<b>4441705.6</b>	<b>1.53</b>
thebaine	31110746	31110766	31007232	31400028	31108074	31442118	<b>31196494</b>	<b>178939.91</b>	<b>0.57</b>
papaverine	187010587	187000798	187665004	187010586	187505898	187665321	<b>187309699</b>	<b>336321.01</b>	<b>0.18</b>



**Table 6.3 %RSD results for pyrolysis products of opium for within-can repeatability extracted in MeOH-DCM 90:10 at 354°C**

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in each ACS in tin can 1								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
meconin	15680908	15680977	15680870	15690060	15685556	15664423	<b>104525744</b>	<b>57546909.31</b>	<b>0.01</b>
hydrocortanine	1823596	1840001	1852236	1822765	1826750	1922143	<b>1847915.167</b>	<b>38112.96868</b>	<b>2.06</b>
codeine	256248560	256248577	258777304	255554097	259978211	262223106	<b>258171642.5</b>	<b>2618886.93</b>	<b>1.01</b>
morphine	13125605	13233212	12899967	13132445	13121769	12902100	<b>13069183</b>	<b>136679.996</b>	<b>1.05</b>
thebaine		154945492	155003424	154900346	154862441	155300218	<b>155002384.2</b>	<b>174598.5893</b>	<b>0.11</b>
papaverine	21648140	21705550	21099760	21671027	20776711	21889220	<b>21465068</b>	<b>429175.0861</b>	<b>1.99</b>
noscapine	115745618	115788801	114998001	115688101	115811994	114977705	<b>115501703.3</b>	<b>400308.4103</b>	<b>0.35</b>

**Table 6.4 %RSD results for opium pyrolysis products for inter-can repeatability extracted in MeOH-DCM 50:50 at 354°C**

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in each tin can								
	can 1	can 2	can 3	can 4	can 5	Can 6	Average	Stdev	%RSD
meconin	62124853	29052810	67053296	1.47E+08	266497648	850093846	<b>236924795</b>	<b>312284714</b>	<b>131.81</b>
hydrocortanine	27829506	5640907	36860987	8155660	136816628	48475460	<b>43963191</b>	<b>48381061</b>	<b>110.05</b>
codeine	190659503	42216046	2.46E+08	3.82E+08	507312603	290338985	<b>276400819</b>	<b>159824800</b>	<b>57.82</b>
morphine	288507145	51453041	1.93E+08	6.21E+08	431877474	501812148	<b>347967663</b>	<b>210024493</b>	<b>60.36</b>
thebaine	31110746	7977150	1.7E+08	1.1E+08	86940133	107219213	<b>85595440</b>	<b>58708506</b>	<b>68.59</b>
papaverine	187010587	75788872	2.42E+08	3.79E+08	524068885	21889220	<b>238219531</b>	<b>188290989</b>	<b>79.04</b>
noscapine	345895760	2.36E+08	3.97E+08	3.63E+08	-	114977705	<b>291454536</b>	<b>115623859</b>	<b>39.67</b>

**Table 6.5 %RSD results for opium pyrolysis products for inter-can repeatability extracted in MeOH-DCM 90:10 at 354°C**

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in each ACS in tin can 1								
	can 1	Can 2	Can 3	Can 4	Can 5	Can 6	Average	Stdev	%RSD
meconin	15680908	21373009	48500646	29035736	21432088	28799678	27470344.17	11481463.44	41.80
hydrocortanine	1823596	11222920	21431106	10788972	11497214	15050685	11969082.17	6389509.185	53.38
codeine	256248560	182332848	171226883	101012317	182379908	179667078	178811265.7	49259725.93	27.55
morphine	13125605	321814742	299588622	163030635	321814742	315204048	239096399	126479351.7	52.90
papaverine	21648140	3450012	4354012	43000876110	8863422	5466381	7174109680	17551460657	244.65
noscapine	115745618	233451	207885	11580943	44536627	33002598	34217853.67	43769344.53	127.91

Table 6.18 %RSD results for pyrolysis products of cannabis for within can analytical repeatability extracted in mobile phase at 354°C

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in tin can 1								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
cannabinol	3212.5212	3212.5212	3212.52	3251.89	3212.51	3213.5611	3220.60	17.497215	0.50
cannabidiol	5303.6406	5303.64	5302.647	5299.112	5301.6201	5202.1233	5285.46	40.863111	0.77

Table 6.19 %RSD results for pyrolysis products of cannabis for within-can analytical repeatability extracted in MeOH-DCM 90:10 at 354°C

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in tin can 1								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
cannabinol	195.6904	195.6097	194.76	195.69	195.6904	194.342	<b>195.2971</b>	<b>0.59</b>	<b>0.30</b>
cannabidiol	112.3989	112.3007	112.3989	112.3899	113.112	112.2877	<b>112.4814</b>	<b>0.31</b>	<b>0.28</b>

**Table 6.20 %RSD results for the pyrolysis products of cannabis for inter-can analytical repeatability extracted in the MeOH-DCM 90:10 at 354<sup>0</sup>C**

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in tin can 1								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
cannabinol	195.6904	127.588	104.336	97.696	171.098	208.786	150.87	47.55	31.52
cannabidiol	112.3989	129.221	208.412	437.712	236.446	386.68	251.81	133.67	53.08

**Table 6.21 %RSD results for the pyrolysis products of cannabis for inter-can analytical repeatability extracted in the mobile phase at 354<sup>0</sup>C**

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in tin can 1								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
cannabinol	3212.521	211.33	284.63	129.77	443.648	313.411	765.89	1098.36	143.41
cannabidiol	5303.641	196.387	256.645	51.371	208.39	165.814	1030.38	1912.09	185.57

Table 6.12 %RSD results for pyrolysis products of cannabis for within can analytical repeatability extracted in MeOH-DCM 50:50 at 354°C

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in tin can 1								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
cannabinol	192817867	192817844	192817866	1.93E+08	1.9E+08	1.93E+08	192349700	1078964	0.56
cannabidiol	337736498	336582956	337765564	3.38E+08	3.38E+08	3.34E+08	336883160	1491169	0.44

Table 6.13 %RSD results for the pyrolysis products of cannabis for within-can analytical repeatability extracted in MeOH-DCM 90:10 at 354°C

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in tin can 1								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
cannabinol	674619964	674619981	674677828	679896320	674619964	67460076	675505804	2151063.6	0.32
cannabidiol	1083886325	1083886732	1082800025	1083886325	108388465	108388642	1083705486	443584.98	0.04

**Table 6.14 %RSD results for pyrolysis products of cannabis for inter-can analytical repeatability extracted in MeOH-DCM 50:50 at 354°C**

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in tin can 1								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
cannabinol	192817867	480750943	319099306	334944305	8057755928	363845896	<b>1624869041</b>	<b>3152806061</b>	<b>194.03</b>
cannabidiol	337736498	767936140	580148840	567220070	136766877956	652239588	<b>23278693182</b>	<b>55597807669</b>	<b>238.83</b>

**Table 6.15 %RSD results for the pyrolysis products of cannabis for the inter-can analytical repeatability extracted in MeOH-DCM 90:10 at 354°C**

Pyrolysis product	Pyrolysis product average peak area from the 6 replicate injections in tin can 1								
	ACS 1	ACS 2	ACS 3	ACS 4	ACS 5	ACS 6	Average	Stdev	%RSD
cannabinol	674619964	480750943	319099306	334944305	8057755928	363845896	<b>1624869041</b>	<b>3152806061</b>	<b>194.03</b>
cannabidiol	1083886325	767936140	580148840	567220070	136766877956	652239588	<b>23278693182</b>	<b>55597807669</b>	<b>238.83</b>